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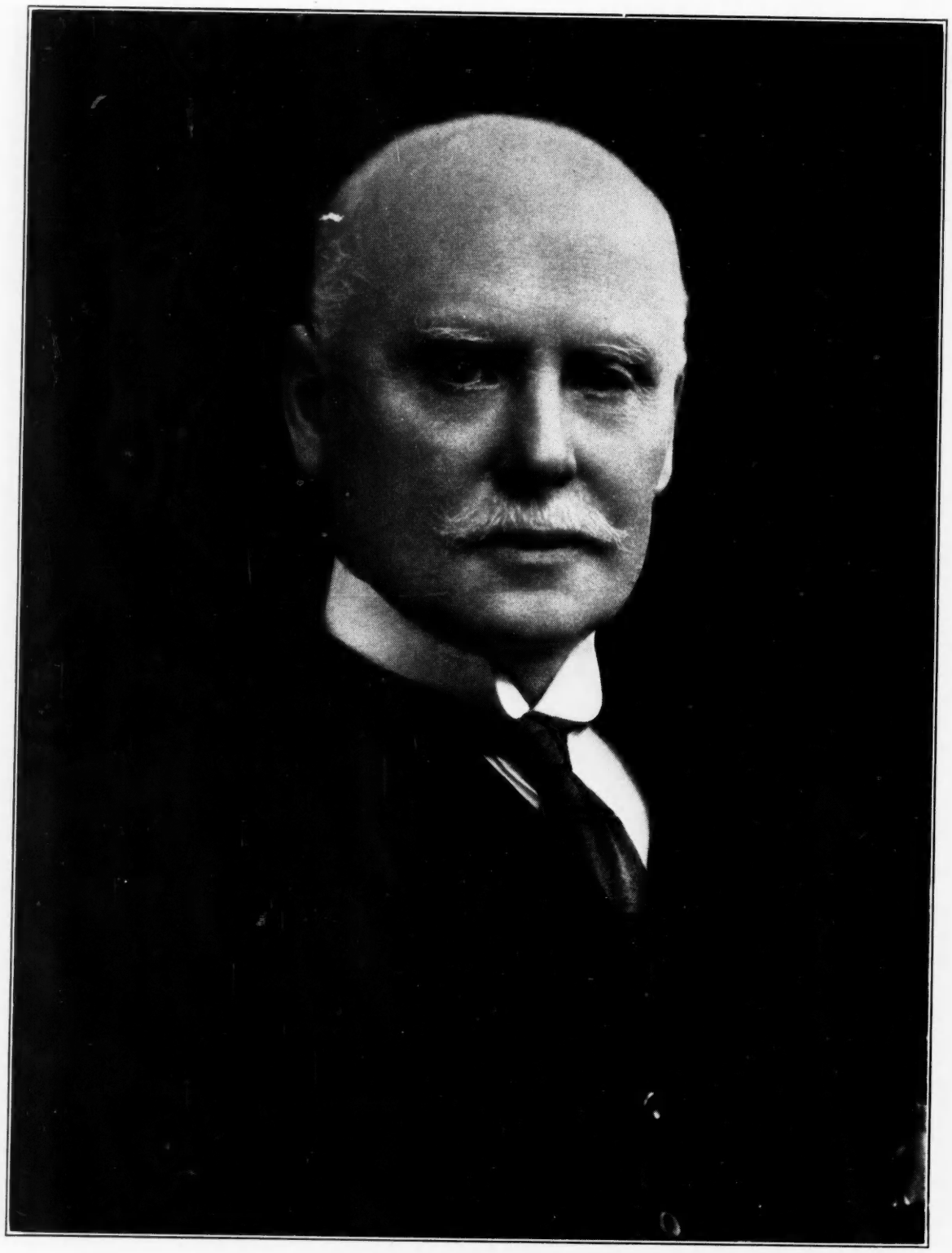

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TRYPANOSOMIASIS OF STOCK IN MAURITIUS*

I. *TRYPANOSOMA VIVAX*, A PARASITE OF LOCAL STOCK

BY

A. R. D. ADAMS, M.D.

(*Received for publication* 29 *December*, 1934)

Trypanosomiasis of domestic stock has been recognized in Mauritius since the introduction of a virulent type of surra into the island in a consignment of bullocks landed in September, 1901, from the Persian Gulf. Up to the present date no trypanosome has been recorded in bovines or equines other than that regarded as *T. evansi*. That the actual date when this parasite is alleged first to have been introduced can be placed with such precision is due to the fact that up till that time cattle for slaughter, draft and, to a lesser extent, for breeding had been imported almost solely from Madagascar; but the Boer War in South Africa created a demand for oxen which Madagascar found it profitable to supply, and thus this island was obliged to turn elsewhere for her regular supplies for a short time. The disease was not at first diagnosed and spread rapidly throughout the island, giving rise to an epizootic which was attended by an enormous and rapid mortality among the local transport animals, bovine and equine. In the period 1902 (July) to 1904 (December) some 5,000 cattle and 4,000 equines perished of the disease, and a recrudescence in virulence of the infection is reported to have taken place in the year 1905 (Laveran and and Mesnil, 1912). The mortality from surra locally at this time was stated among bovines to be from 25 to 30 per cent., while equines once infected invariably died. The above figures for mortality therefore give some idea of the actual incidence of the disease. Parasites were eventually recovered from the serous fluid obtained from oedematous areas on the abdomen of an infected horse by a local medical practitioner, Dr. Aimé Lesur; in due course a diagnosis was reached as to the nature of the condition, treatment was commenced, and the control of all further imported livestock by quarantine methods was instituted. In the meantime, Government had been obliged to come to the assistance of the sugar-planting community—sugar being the staple crop and source of revenue of the colony—as owing to the loss of their animals the planters were unable to harvest their crop; from that time dates the introduction of the steam-tramways, by means of which much of the cane transport is now dealt with on the larger estates. Transport by animal traction, however, is still in considerable use, though the arrival of the motor vehicle has largely banished the horse from the country. Mules and donkeys are to-day employed to some extent, but from the agriculturist's point of view

*From the Bacteriological Laboratory, Medical and Health Department, Mauritius.

they are not as satisfactory as the ox, which greatly predominates as a haulage animal.

During the course of over a hundred years of British occupation of Mauritius many horses and cattle have been imported from other parts of the world, more particularly from Australia and South Africa, though the numbers of these animals can be but a small proportion of those entering this country from Madagascar. African cattle have been used more especially for breeding and improving slaughter-stock and milk-producing herds, and the very efficient model dairy run by the Agricultural Department of the island is stocked with imported South African pedigree cattle and their progeny. Casual importations of such pedigree cattle from Africa by private enterprise have occurred for many years ; and as South Africa is the nearest source of supply for such high-class stock and is also possibly the most suitable, in that the climatic conditions more nearly approach those obtaining locally than would be the case with other stock-raising countries, it follows that in the aggregate over a period of many years large numbers of animals have arrived here from that part of the world.

The actual numbers of the larger domestic animals in the island were determined in 1921, when it was estimated that there were 2,400 equines and 44,339 cattle ; in 1930 it was found that the number of equines had fallen to 1,460 and that of bovines to 31,869. The conclusion reached was that these reductions were due to the increase and improvement in motor traction (Ann. Reps., Dept. Agric., 1920-31). Nevertheless, when it is realized that the island of Mauritius is roughly the size of the county of Surrey, it will be apparent that the concentration of animals is fairly high. The great majority of cattle are used for transport purposes, and are therefore travelling some miles each day on roads and in the fields, more particularly during the crop season, with the result that they come into frequent contact with beasts from other stables. In addition to the draft-oxen, many sugar-estates keep herds of cattle solely for manure production ; these beasts are usually allowed to graze in different parts of the estates during the course of some hours each day, and are then penned into open corrals where the manure is collected. The manure-production herds thus may be in frequent close association with draft-oxen during the travels of the latter. Distinct from either of these two classes of animals are the milch-cattle, which rarely see the full light of day and are kept closely confined in dark stables. This third class of animal is never allowed at large either to graze or to water, but emerges from its dark stables but a few hours on one or two occasions each year when the cows change hands or are taken to the bull. Such cows are kept usually in small numbers, either singly or in twos or threes, all over the island by Indian small-holders, and provide the milk of the country, the natural product generally being liberally supplemented by other fluids to supply the demand. The milch-cattle have for years been deliberately kept in the dark stables referred to above to avoid the attentions

of the ubiquitous *Stomoxys nigra*, which attacks in swarms every large quadruped in the open daylight and almost certainly constitutes the local vector of surra.

Before leaving the subject of the importation of cattle, it is well to emphasize why, apart from its proximity and suitability as a cattle-rearing country, Madagascar is so popular as the source of stock for Mauritius. Wenyon (1926) lists Madagascar as an area where *T. evansi* is found, but this would appear to be an error. I can find no references to trypanosomiasis of stock in that territory in the monthly bulletins of the Madagascar government departments for the last few years. It is a belief in Mauritius that surra has never been found there and, therefore, that cattle imported to this island from that country are quite safe in this respect. The Bulletin Économique (1923) of Madagascar and its dependencies states, under the heading 'Trypanosomiases,'' Grâce aux mesures sanitaires qui interdisent l'entrée des animaux et des fourrages provenant de Maurice et de l'Afrique du Sud, aucun vétérinaire n'a encore jamais trouvé de trypanosomes dans le sang des animaux de Madagascar, sauf chez le rat qui a le *Trypanosoma Lewisi*.' The writer then goes on to remark that a search was made by cultural methods for *T. theileri*, and that even this parasite was never found.

SURRA INVESTIGATION AND CONTROL

In 1921 a campaign against surra was begun by the newly-constituted veterinary branch of the Agricultural Department of Mauritius. It was realized that as an initial step the incidence of the disease should be ascertained, and arrangements were made whereby cattle and draft-oxen, chiefly the latter, should be subjected to examination in various parts of the island. Members of the veterinary staff were empowered to stop oxen on the roads, in the fields, and to enter their stables, and to make blood-films which were later stained and examined for the presence of trypanosomes. Those oxen from which these parasites had been recovered by this means were notified to the local police, who instructed the owners that the animals must be kept in their stables and not allowed abroad until a certificate of satisfactory treatment had been produced. Equines found infected are, by law, destroyed, as it has been regarded as a waste of time and material to treat them. The standard treatment at present employed for bovines consists of intravenous atoxyl and oral arsenious acid. They are reported to respond well to this régime as a general rule, but exceptions occur from time to time which may be of considerable importance in the light of the observations to follow.

In Table I are set forth the figures relating to the numbers of slides examined by the veterinary authorities by the thin-film method, together with the numbers of animals found infected with trypanosomes by this technique. These figures cover a period of the last eleven years, and include all the examinations made since systematic studies of this nature were begun. While numbers of equines found infected are included, it must be remembered that equines are

not examined as a routine, but only when reported sick ; these are usually so ill, and are so susceptible to surra when infected, that single thin-film examinations will probably give a very accurate figure for the infection-rates among them. There can be little doubt but that this is not by any means the case where bovines are concerned, and that even repeated thin-film examinations in the latter might give figures falling short of the true state of affairs. Nevertheless, as a routine and practicable measure with limited staff and facilities, the method has much to commend it as an attempt at diagnosis and control.

In addition to the usual domestic equine and bovine animals a few sheep and pigs are reared in the island, and a large number of goats is kept by the

TABLE I

To show the number of thin-film preparations examined by the veterinary department, and the number of animals found to be infected with trypanosomes during the last eleven years by this means

Year	Total slides examined	Slides found to contain trypanosomes			Percentage of total found so infected
		Cattle	Equines	Total	
1923	5,968	132	18	150	2·51
1924	5,959	31	6	37	0·62
1925	7,330	46	13	59	0·81
1926	7,506	40	4	44	0·59
1927	13,488	60	12	72	0·53
1928	9,675	75	4	79	0·53
1929	7,983	40	3	43	0·54
1930	7,793	24	3	27	0·35
1931	7,826	35	2	37	0·47
1932	7,403	24	3	27	0·36
1933	8,538	28	6	34	0·40

1933a Outbreak among herd of deer.

Indian community. Deer (*Cervus unicolor* var.) were introduced in 1639 from Batavia during the Dutch occupation, and the few animals originally turned down have bred and spread throughout the higher regions of the country districts, until at present it is estimated that there are about 20,000 head. Domestic pigs, also during the Dutch settlement, were allowed to revert to wild life, and are found in the less inhabited tracts of country in large numbers. With the exception of a severe outbreak of surra among a small and localized section of the wild deer (Adams and Lionnet, 1933), trypanosomal infection has not been recorded in any of the above-mentioned animals in Mauritius. This cervine outbreak was studied personally, and parasites, found in wet cover-slip, thick,

and in carefully stained thin films, were seen to conform morphologically to the recognized characters of *T. evansi*. Unfortunately no small-animal inoculations were made, but it was noted at the time that large numbers of the pariah dogs which collected to feed on the numerous carcases lying about were ill and died within a short time. Perhaps it is not inappropriate to mention here that at the time of this outbreak in the deer the possibility of the presence of an African trypanosome was prominently to the fore in the writer's mind, as the many years' association of the deer with surra-infected oxen in Mauritius, and the lack of evidence of any comparable outbreak of the disease among them in the past, were appreciated as being points of very considerable moment in arriving at an opinion of the nature of the virus responsible for this epidemic.

THE ORIGIN OF THE PRESENT ENQUIRY

Early in 1934 a request was received from Dr. C. A. Hoare, of the Wellcome Bureau, for slides showing large numbers of parasites obtained from local strains of *T. evansi*; it was decided that the best way to supply the material would be to inoculate the bloods of a few surra-infected oxen into laboratory animals. Application was accordingly made to the Government Veterinary Surgeon that he would be good enough to inform this laboratory of any cases detected during the routine examination of the large numbers of thin-film preparations undertaken annually by his staff. To this he most kindly assented, and on receipt of notification by him of a case a visit was paid personally to the animal. The beast was cast, 20 c.cm. of blood were withdrawn from a jugular vein, and 10 c.cm. immediately subinoculated into the peritoneum of a guinea-pig, 5 c.cm. into that of another, and 2·5 c.cm. into each of two more. The bloods of these small animals were examined daily for some weeks, and have indeed been watched up to the present date—well over six months later—but no infection developed in any of the three which survived the initial shock of the injection of such large quantities of foreign blood. Thinking that possibly the blood of the ox contained only very scanty parasites at the time of the injections, we found and visited another beast, on this occasion first ascertaining that there were trypanosomes demonstrably present on the day of the proposed visit. Four guinea-pigs were subinoculated as before, and after the inoculations blood was retained in the syringe for subsequent examination at the laboratory, where it was found that about two parasites were present in wet cover-slip preparations per field ($\frac{1}{6}$ in. obj.; 2 oc.). These guinea-pigs also failed to develop any infection and have remained negative up to the present—more than six months later. A third beast was found by the veterinary authorities, and similar subinoculations were made into guinea-pigs with a like result; on examination of the citrated blood at the time, very few parasites were found in cover-slip preparations, so their morphology could not be studied in thin films properly. Yet another infected ox was reported, and, after first determin-

ing that trypanosomes were present on the morning of the projected visit, a journey was made to the stable by my scientific assistant, Mr. Webb, during my temporary absence on leave from the laboratory.

In this stable were six draft-bullocks, all locally-bred animals, and blood was taken from each and examined. Five were found actually to contain trypanosomes on the day of visit, and blood was taken from two of them. From the first of these two, three guinea-pigs were inoculated intraperitoneally with 10 c.cm., 5 c.cm. and 2·5 c.cm. of blood respectively, and of these the first two receiving the larger doses died of shock within two days. The third is still alive and well several months later, and has not become infected. From the second ox, ' Lagueule,' three other guinea-pigs were similarly subinoculated with 10 c.cm., 5 c.cm. and 2·5 c.cm. of blood from a jugular vein. All three animals survived the immediate effects of the heroic doses of foreign blood, but none has shown any sign of infection with trypanosomes, in spite of regular examinations extending over a period of more than five months. On returning to the laboratory, Mr. Webb procured a young goat (6 months), and injected peritoneally a couple of cubic centimetres of the citrated ox-blood remaining in the syringe. Wet cover-slip preparations of this citrated ox-blood were examined at the time, and two or three parasites were to be found in each microscope field ($\frac{1}{6}$ in.); stained thin films had about a trypanosome in every forty fields of the oil-immersion field. This goat, which had received a smaller dose of infecting blood than the guinea-pigs, showed trypanosomes in its peripheral blood on the fourth day from inoculation, and has continued to show varying numbers of parasites until the time of writing. It is with the strain recovered from ox ' Lagueule ' by means of the goat subinoculation that the present paper primarily deals. In Table II are summarized the data relating to the five oxen used unsuccessfully in endeavours to infect guinea-pigs with what had been taken for granted as *T. evansi.*

OX 'LAGUEULE' STRAIN

The ox ' Lagueule ' from which the goat was successfully infected was a particularly fine specimen of a *locally-bred* draft-ox of about six years. It had the general conformation of the large European type of cattle, as opposed to the zebu, and had been reared in another part of the island, and brought to Highlands some three years ago when it was broken to harness. The animal was in very fair condition when trypanosomes were first found several months ago ; it has not been sick, in the ordinary sense of the term, either before or since the infection was detected. The other five animals in the same stable were also all of local breeding, and were in good general condition and perfectly able to continue at their daily work ; all six are to-day working in the fields. All five oxen found to be infected with trypanosomes in this stable were immediately treated by the routine method—atoxyl and arsenious acid—on discovery

of parasites ; none has been ' cured ' of the infection, in spite of repetition on two subsequent occasions of entire courses of the therapy ; trypanosomes may still occasionally be recovered from the bloods of each of them, and at the moment all are in very fair health and condition.

TABLE II

To show the areas in which oxen were found infected with trypanosomes which failed to infect guinea-pigs when inoculated with large amounts of infected ox-blood

Ox	District	Number of trypanosomes per 1/12 in. field of thin films	Animals inoculated	Doses of ox-blood injected peritoneally	Remarks*
I	Curepipe Road	Four in a preparation	Guinea-pig (i)	10·0 c.cm.	Died shock
			,, (ii)	5·0 ,,	
			,, (iii)	2·5 ,,	
			,, (iv)	2·5 ,,	
II	Quatre Bornes	1 in 100	,, (i)	10·0 ,,	
			,, (ii)	5·0 ,,	,,
			,, (iii)	2·5 ,,	
			,, (iv)	2·5 ,,	
III	Candos	1 in 200	,, (i)	10·0 ,,	,,
			,, (ii)	5·0 ,,	,,
			,, (iii)	2·5 ,,	
			,, (iv)	2·5 ,,	
IV	Highlands	1 in 50	,, (i)	10·0 ,,	,,
			,, (ii)	5·0 ,,	,,
			,, (iii)	2·5 ,,	
V	Highlands ' Lagueule '	1 in 40	,, (i)	10·0 ,,	
			,, (ii)	5·0 ,,	
			,, (iii)	2·5 ,,	
			Goat I	2·0 ,,	Strain obtained

*' Died shock ' means that the animal never got over the immediate effects of the inoculation of the dose of ox-blood. All others are alive and well to the present, six months later, and, with the exception of the goat, have never shown the presence of parasites in the peripheral bloods.

After the first two original cases, suspicion was aroused that the trypanosomes with which the inoculations had been made were not, at any rate, typical *T. evansi*, and that paucity of parasites provided a quite inadequate explanation

of the total absence of infection in any one of the guinea-pigs inoculated. A study of the films made originally during the routine examinations when the infections had first been detected did not help matters materially, as the films had been imperfectly fixed and stained with thionin. Study of a few films made from citrated blood at the times of the inoculations and stained with Leishman at the laboratory, resulted in the guarded opinion that the parasites seen in many instances very closely resembled those of the *vivax* group. The goat showed but few parasites in its peripheral blood—insufficient to be studied in thin-film preparations—until the 79th day from its inoculation, when there were at least two trypanosomes in each $\frac{1}{6}$ in. field of a wet cover-slip preparation. A number of films were made and carefully examined on that day, and specimens were sent to Dr. Hoare, of the Wellcome Bureau, and to Professor Warrington Yorke, of the Liverpool School of Tropical Medicine. No reply has had time to reach this rather inaccessible island from either of these gentlemen so far. During the course of the infection in the goat, parasites were usually so scanty that only one or two could be found in the whole of a cover-slip preparation, but on a few occasions, of which that referred to above was an outstanding example, many more trypanosomes were present for a single day only ; on many other days no parasites at all were found after prolonged search through the whole of several preparations. In Table III are set forth data relating to the subinoculations from this goat (Goat I), together with the results obtained in the experiments.

A study of Table III shows that in all 3 dogs, 2 rabbits, 2 white mice, 3 guinea-pigs and an ox (18 months old) were inoculated with blood from Goat I and survived the immediate results of the injections of relatively large doses of foreign blood. Of all these animals only the ox developed a definite and permanent infection, as opposed to the transient infections noticed in a mouse and a rabbit. These transitory infections are of considerable interest, as they have been recorded as having occurred in the dog (Ziemann, 1905), in rabbits and rats (Blacklock, 1912), and possibly in man (Macfie, 1917), but not so far in mice, with *T. vivax* infections. On the face of it, there is nothing to be found in Table III which is inconsistent with the provisional diagnosis of *T. vivax* as the trypanosome recovered from the ox ' Lagueule.'

In Table IV are set out the subinoculations from Ox A, referred to above, which was infected successfully from Goat I. The data are presented in a manner similar to that employed in the previous Table, and it will be observed that none of the smaller laboratory animals has developed any infection after the injection of large doses of heavily infected blood intraperitoneally ; these observations are in complete accord with those made in the attempted passages from the five cattle first seen, and also with the inferences to be drawn from the experimental data presented in Table III.

TABLE III

Showing the results of subinoculation into various animals of large doses of infected blood from Goat I, originally infected from ox ' Lagueule ' on 9.7.34

Date	Animal inoculated	Dose of goat-blood intra-peritoneally	Number of trypanosomes per $\frac{1}{6}$ in. field wet preparation	Remarks*
18.7.34	Mouse 3A	0·2 c.cm.	1 in 2	No infection. Reinoculation on 9.8.34
21.7.34	Dog A	10 „	1 in 100	No infection
26.7.34	Mouse 3B	2·0 „	1 in 2	Trypanosomes in blood on 31.7 (1/100); on 1.8 (1/20); on 2.8 (1/200); none since then
1.8.34	Guinea-pig 4A	10·0 „	1 in 2	Died shock
	„ 4B	5·0 „	„	No infection
	„ 4C	2·5 „	„	„
	„ 4D	2·5 „	„	„
	Rabbit 5A	10·0 „	„	„
	„ 5B	10·0 „	„	Trypanosomes in blood on 9.8 (1 seen); on 10.8 (1/80); since negative for three months
9.8.34	Mouse 3A (Reinoculation)	1·0 „	1 in 4	No infection
25.9.34	Dog B	10·0 c.cm.	2 in 1	No infection
	„ C	10·0 „	„	„
17.10.34	Ox A†	20·0 „	1 in 100	Trypanosomes in blood on 24.10 (1/10); on 25.10 (1/1); on 26.10 (5/1); and up to present, a month and a half later, trypanosomes are daily present in varying numbers

†The blood of Ox A was examined daily for two weeks before the animal was used; no trypanosomes were found in it in that time.

*'1 seen' means that a single parasite was found in a whole cover-slip preparation. '1/10' means that one parasite was present in each 10 $\frac{1}{6}$ in. fields of the microscope; and so on.

TABLE IV

Showing the results of subinoculation into various animals of large doses of heavily infected blood from Ox A, infected on 17.10.34 from Goat I with ox ' Lagueule ' strain *T. vivax*

Date	Animal inoculated	Dose of ox-blood intraperitoneally	Number of trypanosomes per $\frac{1}{6}$ in. field	Remarks
24.10.34	Mouse 5A	2·0 c.cm.	1 in 10	No infection
	„ 5B	2·0 „	„	„
	Dog D	10·0 „	„	„
	„ E	10·0 „	„	„
	Monkey I	10·0 „	„	„
	Rabbit 6A	10·0 „	„	„
	„ 6B	10·0 „	„	„
	„ 6C	10·0 „	„	„
	„ 6D	10·0 „	„	„
	Guinea-pig 7A	10·0 „	„	Died shock
	„ 7B	5·0 „	„	No infection
	„ 7C	5·0 „	„	Died shock
	„ 7D	5·0 „	„	No infection
	„ 7E	5·0 „	„	„
	„ 7F	5·0 „	„	Died shock

MORPHOLOGY OF THE STRAIN AND ITS IDENTIFICATION

As already stated above, the slides originally made from the bloods of the cattle used for inoculation of small animals in the field, which had been prepared during the course of the routine investigation into the incidence of surra in the colony, proved of little value for an exact morphological study. Slides made at the laboratory from the blood of ox ' Lagueule ' and its four stable-companions showed relatively few parasites in stained thin-film preparations. Later examinations of the bloods of these beasts also failed to yield sufficient trypanosomes to permit of a proper and satisfactory study of this nature, although the opinion had early been reached that the morphology of isolated trypanosomes closely resembled that of the *vivax* group. Until confirmatory animal infections had been obtained with the resultant hoped-for heavy infestations it was felt inadvisable to venture any open expression of opinion in view of the present position of knowledge as to the distribution of this group of trypanosomes. The earlier possibility of the strain's being one of *T. vivax* was confirmed to a considerable extent by careful study of films made from the goat, although it was not until the 79th day of the infection in this animal that sufficient trypanosomes were present in its blood for a full and critical examination ; this

examination left no doubt that the provisional tentative diagnosis was correct, and that the parasite was definitely *T. vivax*. Further films made subsequently from the young ox (Ox A) subinoculated from the goat amply confirmed the finding, as there were plenty of trypanosomes to be found in thin-film slides within the first few days of the infection in the beast.

The detailed morphology of the strain, when it could properly be studied, was sufficiently striking to leave no further possible doubt in the writer's mind that the trypanosome conformed to the *vivax* group in all major details (fig. 1). The monomorphic form, free flagellum, and characteristic shape became immediately apparent when large numbers of well-stained parasites

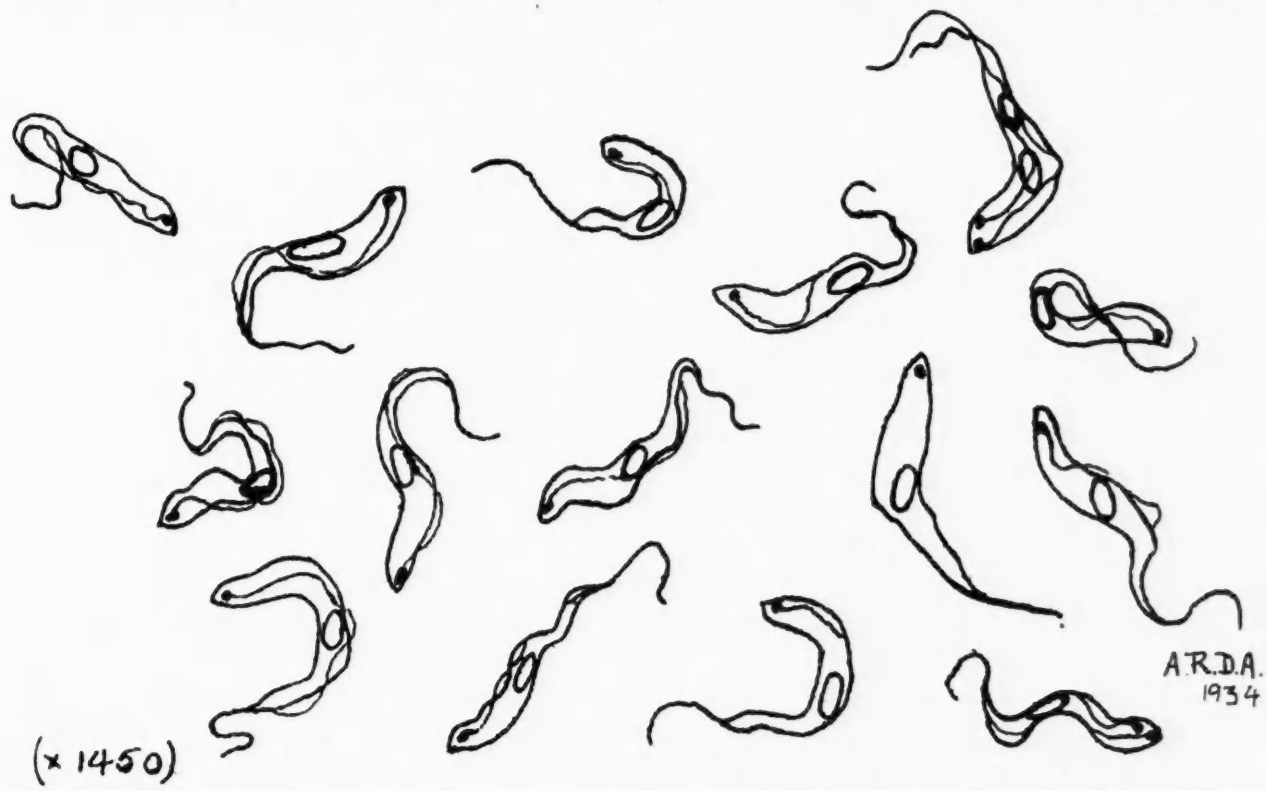

FIG. 1. Camera lucida drawings of trypanosomes in blood of Ox A, infected with *T. vivax* from Goat I, which had been infected from Highlands ox 'Lagueule,' experimentally by syringe-inoculation.

could readily be found in a single thin-film preparation, and these characters, combined with the large almost terminal blepharoplast, the rather straight axoneme, and the narrow undulating membrane, pointed to only one possible identification, assuming that the parasite belonged to a known and not to a new species of the genus *Trypanosoma*. While detail measurements of large numbers of trypanosomes would doubtless prove of value in establishing the diagnosis and in determining the race of *T. vivax* to which this parasite might be assigned, the time available for purely systematic work of this nature is small in a laboratory with limited staff dealing with a large daily routine of general medical work. The motility of the trypanosomes in the goat did not appear as great —in so far as translatory motion was concerned—as it was later found to be in the

ox ; in the latter animal translatory movement was seen to advantage, and was appreciably greater than that to be observed in a strain of *T. evansi* later recovered and used for comparison.

Taking into consideration morphology, motility, the possibilities of importation into the country and, above all, infectivity for laboratory animals, the inevitable conclusion is reached that the strain recovered from ox ' Lagueule ' by goat-inoculation is one of *T. vivax*. This parasite was almost certainly the trypanosome present in the four animals previously used for guinea-pig inoculations, and thus the inference is that the infection is already established and disseminated in the island, and is not confined to a single isolated stable.

RECOVERY OF A LOCAL STRAIN OF *T. EVANSI*

In confirmation of the conclusions already reached as to the nature of the ox ' Lagueule ' strain it became advisable to continue even more vigorously a search for *T. evansi*, so that this might be studied both to confirm its actual existence and to serve as a control on the observations already made with the previous strain. The very next animal reported as being infected with trypanosomes by the veterinary staff provided the desired material. The ox (Long Mountain ' Sam ') was another locally-bred ' creole ' ox from an estate some fourteen miles distant from any of those already seen. A visit was paid to the beast immediately on receipt of the information ; 20 c.cm. of blood were withdrawn from a jugular vein, 10 c.cm. inoculated peritoneally into one guinea-pig, 5 c.cm. into another, and 2·5 c.cm. into each of two more. A further supply of blood was drawn, citrated, and put in a tube ; thick and thin films were made on the spot from the blood of the ox, and were examined on return to the laboratory. The morphology of the parasite (fig. 2) was found to be characteristic

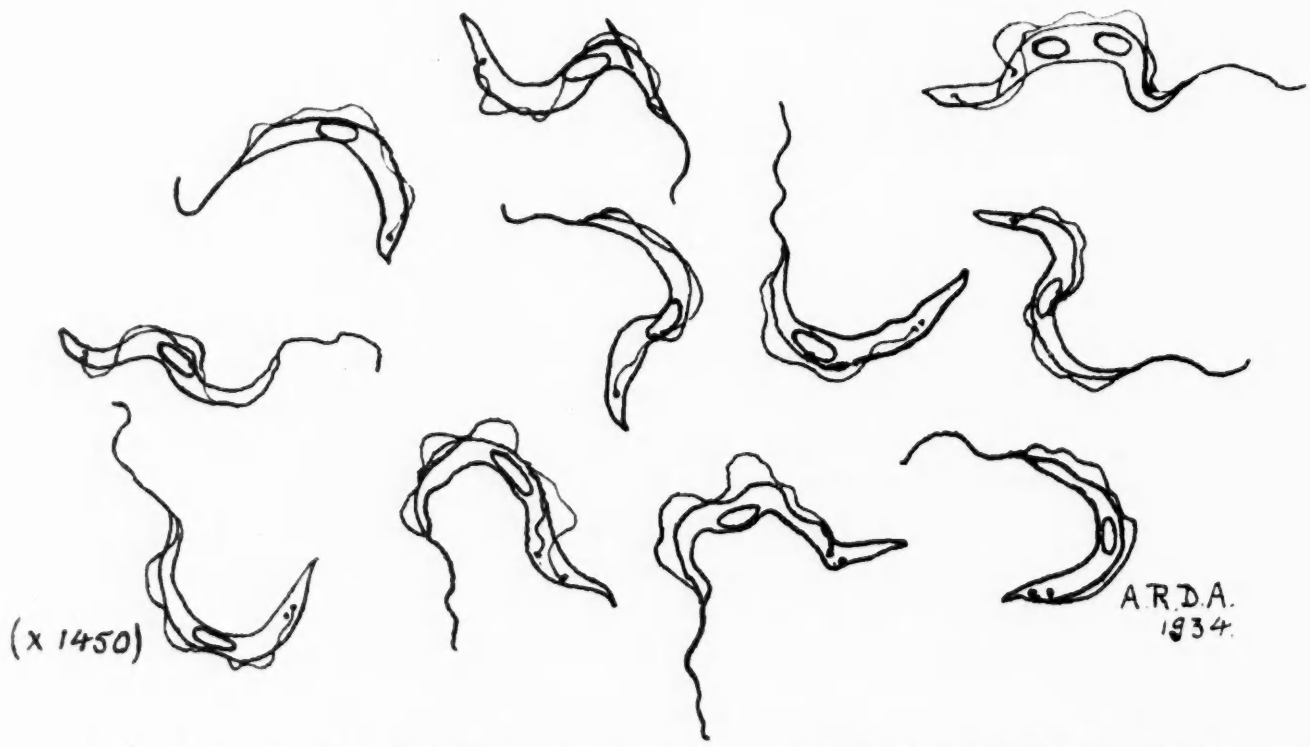

FIG. 2. Camera lucida drawings of trypanosomes in blood of ox Long Mountain ' Sam,' infected with *T. evansi* naturally in the field.

of that of *T. evansi*, and 2 rabbits, 2 dogs, a monkey (*Macacus cynomolgus*), a white mouse and further guinea-pigs were inoculated intraperitoneally with relatively small quantities of the citrated blood. Parasites were found to be present in this citrated blood in comparatively large numbers in wet cover-slip preparations, five to ten being seen in each microscope field ($\frac{1}{6}$ in.). Many could be found with ease in thin-film slides stained with Leishman, and the morphology was typical of *T. evansi* in these. In Table V are set forth the

TABLE V

To show the infectivity and virulence of a strain of *T. evansi* from a local ox (Long Mountain 'Sam') to small laboratory animals

Date	Animal inoculated	Dose of ox-blood intra-peritoneally	Number of trypanosomes* per $\frac{1}{6}$ in. field	Remarks
27.10.34	Rabbit 8	0·5 c.cm.	15 in 1	1 in 4 on 30.10; 1 in 5 on 31.10; and present in varying numbers almost daily until present 19.11.34
	Guinea-pig 9A	10·0 „	„	Died shock 6 hours
	„ 9B	5·0 „	„	„ 18 „
	„ 9C	2·5 „	„	1 in 50 on 29.10; 1 in 5 on 30.10; and present in large numbers daily till death on 5.11.34
	„ 9D	2·5 „	„	1 in 20 on 29.10; 1 in 10 on 30.10; 3 in 1 on 31.10; and present almost daily until present 19.11.34
	Mouse 10B	0·25 „	„	Numerous parasites on 29.10; swarming until death on 1.11.34
	Monkey 3 (*Macacus (cynomolgus*)	0·25 „	„	1 in 20 on 29.10; numerous and finally swarming till death on 5.11.34
	Dog A (Already used	0·5 „ on 21.7.34; see	„ Table III)	1 in 30 on 29.10; 1 in 2 on 30.10; then numerous and swarming till death on 16.11.34
	Puppy F	0·15 c.cm.	15 in 1	1 in 1 on 29.10.34; and then swarming till death on 9.11.34

*Wet cover-slip preparations.

details of subinoculations with the blood of this ox, and a cursory study of this Table will immediately show that the results obtained are those expected of *T. evansi* of normal virulence, and are very different from the results obtained with the ox ' Lagueule ' strain ; all the subinoculated animals became infected within two or three days, and all died of the infections within a week or two after having consistently shown large numbers of parasites in the peripheral bloods. The morphology of the trypanosomes recovered from each of these animals was characteristic of the injected strain.

There is thus no further doubt that at least two entirely distinct trypanosomes are present in local stock ; and, in addition, that each infection may be acquired locally in the island, as each of the two animals from which representative strains have been isolated has been bred and reared in the colony. That each parasite is widespread in the country is also evident, in that what is regarded as *T. vivax* was found in five beasts each coming from a stable separated by some miles from the others ; and that *T. evansi* has been causing undoubted exacerbations of surra in stables all over the island for many years past.

POSSIBLE LOCAL INSECT VECTORS OF *T. VIVAX*

Representatives of the genus *Glossina* are entirely absent from Mauritius, and are exceedingly unlikely ever to be introduced here by shipping. There is little doubt but that, if they were introduced intentionally, several species would flourish.

Stomoxys nigra is a ubiquitous pest throughout the island where it is colloquially known as ' mouche boeuf,' or ' cane-fly.' It attacks in large numbers all the larger mammals, and its attentions are such to man that overseers in the fields wear leather gaiters as a protection from its activities ; cattle and equines at work are usually partially protected by sacking and leafy branches of trees, and by switches wielded by their drivers. Smudge fires are frequently lighted by thoughtful drivers to protect their beasts during rests in the fields or on the roads, an attention much appreciated by the animals. On many estates the cattle are all passed through a Bourgault fly-trap (Loughnan, 1930) two or three times a day, a means of relief which the oxen are very ready to seek voluntarily.

Hippobosca maculata is found, but is local and concentrated in its distribution. This insect is thought to have been introduced with cattle consignments from Madagascar, and has established itself in certain localities in the island. *Tabanus ditaeniata* also occurs, but is very rare and found only on the open hills, never near stables. It is apparently not a blood-sucking species and consequently is of no importance in the present enquiry. No species of *Haematopota* is known to exist in Mauritius.

It would thus seem very probable that the most likely vector of the trypanosomes of stock is *Stomoxys nigra* ; and that it may so act was to some degree demonstrated locally by Moutia (1928), with what was regarded at the time of his work as *T. evansi*.

PREVIOUS RECORDS OF THE ESTABLISHMENT OF AFRICAN TRYPANOSOMES IN COUNTRIES DEVOID OF *GLOSSINA*

Leger and Vienne (1919) found and described a trypanosome in French Guiana which they thought to be a new species and named *T. guyanense*. The parasite was studied by a few workers who, chiefly on morphological grounds, agreed that it was probably a member of the *vivax* group. The matter received attention at the hands of one or two workers, but does not appear to have been pursued very thoroughly, and there seems to exist a certain doubt as to the authenticity of the record in default of fuller information.

Stirling (1921) reported the presence of a trypanosome in a bullock in the Central Provinces of India which had the characters of *T. congolense*.

In Zanzibar, Aders (1923) recorded that *T. congolense* was being spread in the island in the absence of *Glossina*, most probably by a species of *Tabanus* chiefly ; but in spite of the regular importations of most of the various trypanosomes of stock from the neighbouring East African coast ports none of the other species of these parasites had gained a footing in the island as far as his investigations pointed.

Apart from these few instances there does not seem to be any record of the pathogenic trypanosomes of stock, normally undergoing a cycle in *Glossina* spp., becoming established and spreading in countries devoid of the usual insect vectors.

THE IMPORTANCE LOCALLY OF THE PRESENCE OF *T. VIVAX*

The importance to Mauritius of the discovery of the presence of *T. vivax* in stock lies in the fact that two entirely distinct trypanosomal diseases are in the island, in place of the single one hitherto known as surra. Surra would appear to have established itself in the colony some thirty years ago, and is apparently typical of surra elsewhere. It is a fatal disease to equines ; bovines infected with it at best lose condition, and there is a definite mortality associated with it in these animals if untreated. *T. vivax* infection has hitherto been unrecognized, so that its duration in the island is a matter of speculation. Its virulence and general effects on local stock have not yet been ascertained accurately, but they would appear to be less than might be expected where the parasite is propagated by its normal insect vectors. This view rests on the experimental inoculation of a goat and of a young ox, as far as these experiments have proceeded, and also on the observation over a period of many months of several cattle found infected in the field. It is premature to make assertions as to its probable distribution, virulence, methods of spread and treatment until fuller information is available. To the stock-owner at the moment the most important aspect of the situation centres on specific diagnosis of all cases of trypanosomiasis discovered in his animals. If the parasite in his stock proves to be *T. evansi* he knows that treatment of bovines is reasonably safe and efficacious if properly carried out. If it proves to be *T. vivax* it is a very different matter. The five oxen in

Highlands stable found infected with *T. vivax* were immediately given a course of arsenious acid and atoxyl ; in every case trypanosomes were found some days after the course in the blood ; two further full courses of therapy were administered to each animal with similar results ; trypanosomes may still be recovered occasionally from each animal ; and all are at work and seemingly in very fair health.

In addition to the diagnosis and treatment of infected bovines, the future position of equines comes prominently to the fore. At present there are rigid regulations regarding the destruction of equines found infected with ' surra,' which may require some modification in the light of further knowledge. A *T. evansi* infection will kill equines with certainty if untreated, and even if successfully treated is responsible for a crippling disability leaving many after-effects. Slaughter has been regarded as a method of cutting short suffering, and also as a prophylactic sanitary measure to lessen the likelihood of dissemination of what is regarded locally as a particularly virulent strain of virus. If *T. vivax*, as is probably the case, is neither fatal nor crippling to equines, slaughter is hardly justifiable on either humanitarian or prophylactic grounds, more especially while similarly infected bovines are allowed at large.

Before anything else is done a full investigation of the position of the local strains of *vivax* is imperative. The general incidence and virulence for all forms of local stock must be determined in the interests of the stock-owners ; and on the results of this investigation will be founded a fuller and more enlightened appreciation of the situation. Suggestions for means best suited to deal with it can be put forward on the facts as they exist and not on purely hypothetical considerations.

I am exceedingly grateful to the Director of the Medical and Health Department, Dr. J. Balfour Kirk, for his encouragement in the pursuit of a subject rather outside the narrower sphere of the departmental laboratory ; also to the Government Veterinary Surgeon, Dr. Lionnet, and his staff for their most helpful co-operation in the matter of information and material. To my able scientific assistant, Mr. Lewis Webb, I am greatly indebted for his enthusiasm and energy throughout the whole course of the work.

SUMMARY

1. Numerous cattle and equines have been imported into Mauritius from all parts of the world during the hundred years or so of British occupation.

2. The great majority of the cattle have for many years past come from Madagascar, where trypanosomiasis of stock does not exist. Numbers of better-class animals have periodically arrived from South Africa and, to a lesser extent, from other cattle-raising countries.

3. Surra was probably imported into the country from the Persian Gulf in a consignment of bullocks landed in the island in September, 1901.

4. This disease immediately and rapidly spread throughout the colony, giving rise to an epizootic which was accompanied by an enormous mortality among both bovines and equines. A serious situation was created in the local sugar industry, as the sugar-crop could not be harvested owing to the loss of transport animals.

5. Measures were taken, when the diagnosis of the condition had been established, to treat infected bovines and to destroy infected equines. Quarantine regulations were drawn up to deal with all future importations of live-stock.

6. The disease is endemic at present, and a fresh campaign against it was begun by the veterinary section of the Department of Agriculture in 1921. The methods employed are briefly stated.

7. No trypanosomes, other than that regarded as *T. evansi*, have ever been recognized in stock in this country up to the present.

8. Strains of *T. evansi* were wanted by this laboratory, and a request was made to the veterinary authority for information of detected cases of surra. The veterinary staff very kindly agreed to inform the laboratory of all future cases found by them in their routine examinations.

9. The first five oxen reported by them were visited, and blood from each was inoculated into a number of guinea-pigs without producing any infection.

10. The morphology of the scanty trypanosomes seen in slides from several of these animals was noticed to conform to that of the *vivax* group of trypanosomes.

11. From the fifth beast a young goat was successfully inoculated with a very small dose of blood. The strain recovered from this goat was readily transmitted to a young ox, but would not infect any of a series of smaller laboratory animals.

12. Morphologically, in its infectivity for animals and with its motility, the trypanosome was shown to conform to all the characters of *T. vivax*.

13. The four stable-companions of this ox from which the strain was recovered were found to be infected with a morphologically similar trypanosome.

14. All the nine animals already found to contain parasites of this type were locally bred, and were housed in five stables each some miles from the others.

15. A tenth beast reported as being infected with trypanosomes was found to be infected with typical *T. evansi*, in so far as morphology and infectivity for small animals was concerned.

16. It is therefore apparent that at least two trypanosomes occur in local stock ; that each is widespread in the island ; and that each is acquired locally, as all the animals examined were locally bred.

17. A brief summary of previous records of the establishment of one of the African trypanosomes of stock in the absence of its normal vectors, *Glossina* spp., is given.

18. The commoner biting insects present in Mauritius are listed ; among

these is probably the local vector of both these trypanosomes, and the evidence points to *Stomoxys nigra* as the most likely culprit.

19. The fundamental importance of *specific* diagnosis in cases of trypanosomiasis of stock in the colony is emphasized, as is also the obvious immediate necessity for fuller detailed investigation into distribution, virulence and methods of treatment, before either the present regulations dealing with animal diseases are modified or further regulations are promulgated to deal with the situation.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE GENERA OF THE SUBFAMILY CALLIPHORINAE BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

THE GENUS CALLIPHORA ROBINEAU-DESVOIDY (sens. lat): A PRACTICAL GUIDE TO THE AUSTRALIAN SPECIES

(*Continued from Vol.* 28, *page* 216)

BY

W. S. PATTON

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 14 *January*, 1935)

INTRODUCTION

The Australian species of the genus *Calliphora* are of peculiar interest, for the majority of the species are, unlike their European allies, of a yellowish or golden colour, and until recently it was believed that there were only about half a dozen species. Mr. G. H. Hardy, the well-known authority on the Australian Diptera, using the characters of the male terminalia, together with his unique knowledge of the species, has, however, succeeded in separating many others, whose identities had been obscured by their close similarity to well-known ones. Mr. Hardy has been collecting series of both sexes of the species known to him, and has very generously placed them at my disposal for a more exhaustive critical study of the ♂ and ♀ terminalia. Mr. Taylor, of the School of Public Health and Tropical Medicine, Sydney, has also very kindly sent me a very large collection, which contains many specimens collected by Mr. N. J. B. Plomley and Mr. T. G. Campbell. This collection is of special interest as it contains some specimens of Mr. J. R. Malloch's species, identified by him, and affords a good opportunity of settling their identities. Dr. Nicholson and Dr. Mackerras have also kindly given me many named specimens from their collections. I also have material from Mr. W. W. Froggatt and Mr. Hill. I wish to take this opportunity of thanking these gentlemen for the great trouble which they have taken in placing this material at my disposal. A list of species (with localities), further specimens of which will enable me to complete this guide, is given at the end of this paper.

Before describing the species it is necessary to understand their natural grouping and relationships as known at present. I have so far been able to study the terminalia of 15 species, which fall into three natural groups as follows :—

Augur Group	Canimicans Group	Erythrocephala Group
augur F.	*canimicans* Hardy	*erythrocephala* Meig.
nociva Hardy	*sternalis* Malloch	*australis* Boisd.
centralis Malloch	*fuscofemorata* Malloch	*ochracea* Schiner
fallax Hardy		*stygia* F.
fulvicoxa Hardy (*albifrontalis* Malloch)		
rufipes Macq. (*hilli* Patton ; *varifrons* Malloch)		
tibialis Macq.		
australica Malloch		

Mr. Hardy has drawn my attention to the fact that my *hilli* is identical with *rufipes* Macq., and I can confirm this conclusion as a result of the study of the male terminalia ; *hilli*, therefore, becomes a synonym of *rufipes*. A study of the ♂ terminalia and the males of *fulvicoxa* and *albifrontalis* leaves no doubt that they are identical, and the latter name must, therefore, be sunk as a synonym of the former. Similarly, I find that *varifrons* Malloch is identical with *rufipes* Macq. Mr. Hardy has suggested to me that the ♂ terminalia of *fallax* are indistinguishable from those of *rufipes* ; the ♀ terminalia too are very alike. As I have not sufficient material to reach a final conclusion I am keeping them distinct at present. The ♂ and ♀ terminalia of *augur* and *nociva* Hardy are also indistinguishable ; but for other reasons Mr. Hardy considers that they are distinct ; my material is too limited to express a final opinion.

In order to understand the above grouping, it is most important to know the structure of the ♂ and ♀ terminalia of *erythrocephala* and its allies. The ♂ terminalia of four typical species (*erythrocephala*, *vomitoria*, *viridescens* and *coloradensis*) have already been described (1934), and the reader should consult that paper and study the drawings. The ♀ terminalia of two of these species (*erythrocephala* and *vomitoria*) are illustrated in this paper. The ♂ and ♀ terminalia of a typical example (*augur*, *canimicans* and *ochracea*) of each of the above three groups will now be described, so that in subsequent papers it will be an easy matter to place a species in its group. Opportunity is taken here of describing the terminalia of *C. grahami* Aldrich. As the various parts are fully illustrated (drawn to scale), the descriptions will be as short as possible. In all these species in the ♂ tergum 6 is more or less well developed (except in the species *sternalis*, in which it is much reduced), but does not extend laterally. Similarly sternum 6 is more or less complete, extending round to the right (in *sternalis* it is very wide and has a deep flange). These sclerites, therefore, will not be described. As it is very difficult to be quite sure of the shape and extent of the sclerites of the ovipositor when fully extended and mounted on a slide in Canada balsam, they have been dissected off and drawn separately at the sides in each case.

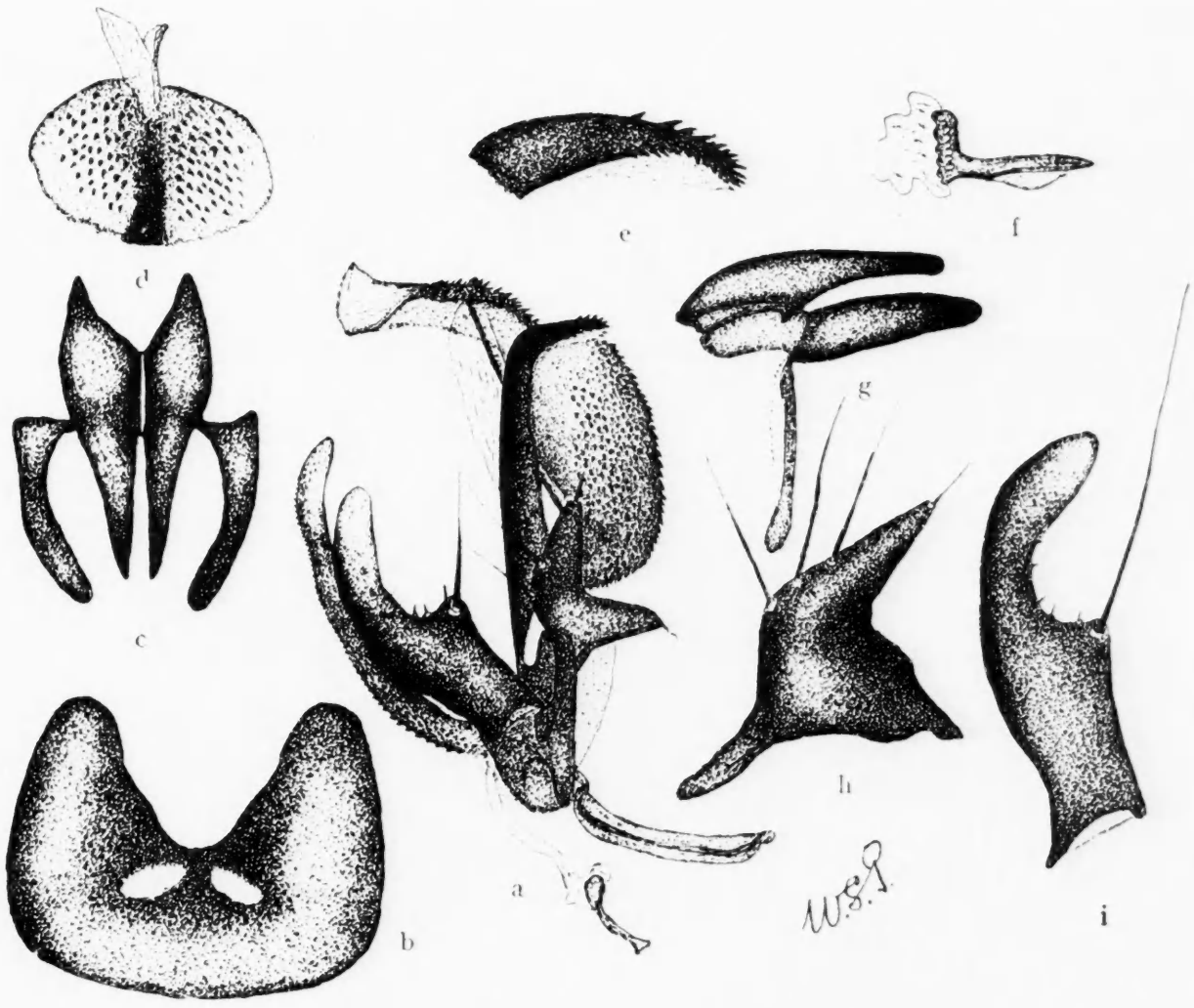

FIG. 1. *a.*—Phallosome and one paramere of *C. augur* in side view; *b.*—Fifth sternum of same; *c.*—Ventral view of anal cerci and distal segment of ninth coxites of same; *d.*—Expanded end of phallosome of same; *e.*—End of strut of same, showing serrations; *f.*—Sperm pump sclerite of same; *g.*—Lateral view of anal cercus and ninth coxite of same; *h.*—Anterior part of right paramere of same; *i.*—Posterior part of right paramere of same (Brisbane specimens).

AUGUR GROUP

Calliphora augur F. SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. SCLERITES. Tergum 5 as in fig. 1, *b*. APPENDAGES. *Ninth Coxite. Distal Segment.* Ventral view. Fig. 1, *c*. Short, but longer than anal cercus, end rounded and turned inwards. Lateral view. Fig. 1, *g*. Broad at base, narrowing towards end. *Proximal Segment.* Lateral view. Fig. 1, *g*. A rather short, wide rod joined to its fellow by chitinized membrane. *Anal cercus.* Ventral view. Fig. 1, *c*. A short, sharply-pointed rod, more than the anterior half free, remainder joined to fellow. Lateral view as in fig. 1, *g*. *Phallosome.* Lateral view. Fig. 1, *a*. Broad, the proximal portion relatively long; the middle part with a pair of stout struts, their pointed ends bent dorsally and heavily serrated (fig. 1, *e*); the dorsal membranous part long and wide, and heavily armed with short, backwardly directed spines; the distal part of phallosome

bearing the ejaculatory duct expanded like the hood of a cobra, with numerous fine spines, and supported by a median chitinous rod, the duct opening on an elongated portion (fig. 1, *a*, *d*). *Posterior Process of Phallosome.* Lateral view. Fig. 1, *a*. Exceptionally long and narrow, its posterior face and sides armed with numerous spines. *Sperm Pump Sclerite.* As in fig. 1, *a*, *f*. *Apodeme of Phallosome.* Lateral view. Fig. 1, *a*. Very short. *Parameres.* Lateral view. *Anterior Part.* Fig. 1, *a*, *h*. Rather short, broad, somewhat acutely bent upwards, bluntly pointed and armed with 3-4 short hairs (one apical) along ventral curved border. *Posterior Part.* Fig. 1, *a*, *i*. A stout upstanding plate, the rounded narrow end widening basally to form a shelf on which a long stout hair is attached.

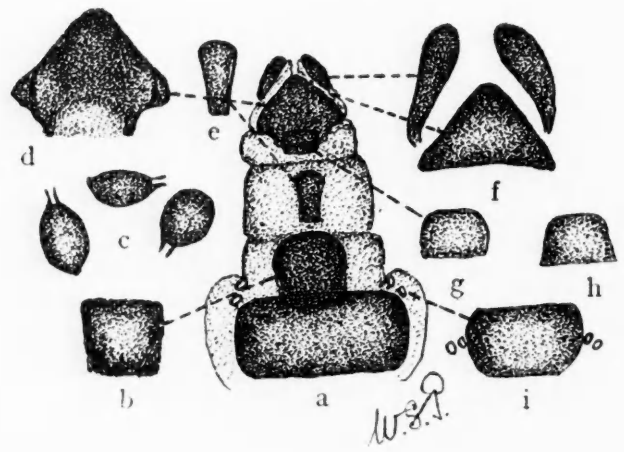

FIG. 2. *a*.—Ventral view of fully extended ovipositor of *augur* ; *b*.—Sixth sternum of same ; *c*.—Spermathecae of same ; *d*.—Tenth sternum of same ; *e*.—Seventh sternum of same ; *f*.—Tenth tergum and anal cerci of same ; *g*.—Ninth sternum of same ; *h*.—Seventh tergum of same ; *i*.—Sixth tergum of same (Canberra specimen).

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. Fig. 2. The fully extended ovipositor is illustrated in fig. 2 ; its striking feature is that it is very short (cf. fig. 2, *a*, with fig. 6, *a*, which is that of *erythrocephala* drawn to the same scale). As the various sclerites and spermathecae are illustrated at the sides it is unnecessary to describe them. It should be noted that the sclerites in this species are very lightly chitinized and that it is difficult to be quite sure of their exact extent. In a subsequent paper I shall draw attention to the characters of the ovipositor of use in the identification of the ♀ *augur*.

It will be noted in subsequent papers that the phallosome in the seven other species of this group known at present is structurally similar to that of *augur*. In all species the struts are stout, heavily chitinized rods, commonly bent upwards at their ends, which are serrated and not expanded at the end, as they are in the much slenderer struts of the phallosome of the *erythrocephala* group. The dorsal membranous flaps are much longer and wider, and the

ejaculatory duct opens at the end of an expanded membrane, strengthened by some chitin and spines (cf. fig. 1, *a*, with fig. 4, *a*). The anal cerci and ninth coxites do not afford group characters, as they vary in the species. The ovipositor of *fallax*, *rufipes*, *fulvicoxa*, *centralis* and *nociva* is also short, adding confirmation to the relationships based on the structure of the phallosome. It is interesting to note that *centralis* is larviparous, for numerous larvae have been found in the abdomen of ♀♀. Does it deposit its larvae in the nest of some burrowing animal or in the nest of a bird, and is the larva a blood-sucker ? I hope some Australian observer will investigate its life-history. So far as I can say at present, *C. australis* Malloch appears to be a link between the *augur* and the *erythrocephala* groups, and *C. sternalis* Malloch to be a link between the *augur* and *canimicans* groups.

CANIMICANS GROUP

Calliphora canimicans Hardy. SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. SCLERITES. Tergum 5 as in fig. 3, *f*. APPENDAGES. *Ninth Coxite*. *Distal Segment*. Ventral view. Fig. 3, *e*. A rather short rod, narrowing towards end, which is characteristically bent upwards (fig. 3, *b*), and longer than anal cercus. Lateral view. As in fig. 3, *b*. *Proximal Segment*. Lateral view. Fig. 3, *b*. A long stout rod joined to its fellow by chitinized membrane. *Anal Cercus*. Ventral view. Fig. 3, *e*. A strong and (in this view) pointed rod, its anterior half free. Lateral view. Fig. 3, *b*. In this view it appears almost as wide as the distal segment of the ninth coxite. *Phallosome*. Lateral view. Fig. 3, *a*. Relatively short and narrow, the proximal part long, as in *augur* ; middle portion with a pair of stout struts, narrowing for a short distance near their ends, which are turned upwards and end in sharp points. The anterior spined membranous flaps are much reduced, and the part between them is strongly chitinized, especially distally, sharply folded back, so that the distal portion carrying the duct is bent cup-like into the end of the phallosome, and the distal part is, therefore, shortened ; the opening of the duct projects at the ventral end of the phallosome (fig. 3, *a*). *Posterior Process of Phallosome*. Lateral view. Fig. 3, *a*. Long, narrow and not spined. *Sperm Pump Sclerite*. As in fig. 3, *a*. *Apodeme of Phallosome*. Lateral view. Fig. 3, *a*. Long. *Parameres*. Lateral view. *Anterior Part*. Fig. 3, *a*, *d*. A rather short plate armed with 4–6 hairs on curved ventral border. *Posterior Part*. Fig. 3, *a*, *c*. A broad, upstanding plate, the distal end wide, narrowing about middle to form a shelf (as in *augur*) on which a very long bristle is attached. I am not able at present to describe the female terminalia.

As will be seen in subsequent papers, the phallosome in the two other species of this group is structurally similar to that of *canimicans* ; *fuscofemorata* Malloch is closely related to *canimicans*. *C. sternalis* Malloch, on the other hand, is a link between this and the *augur* group. It is of peculiar interest, for it has a strong

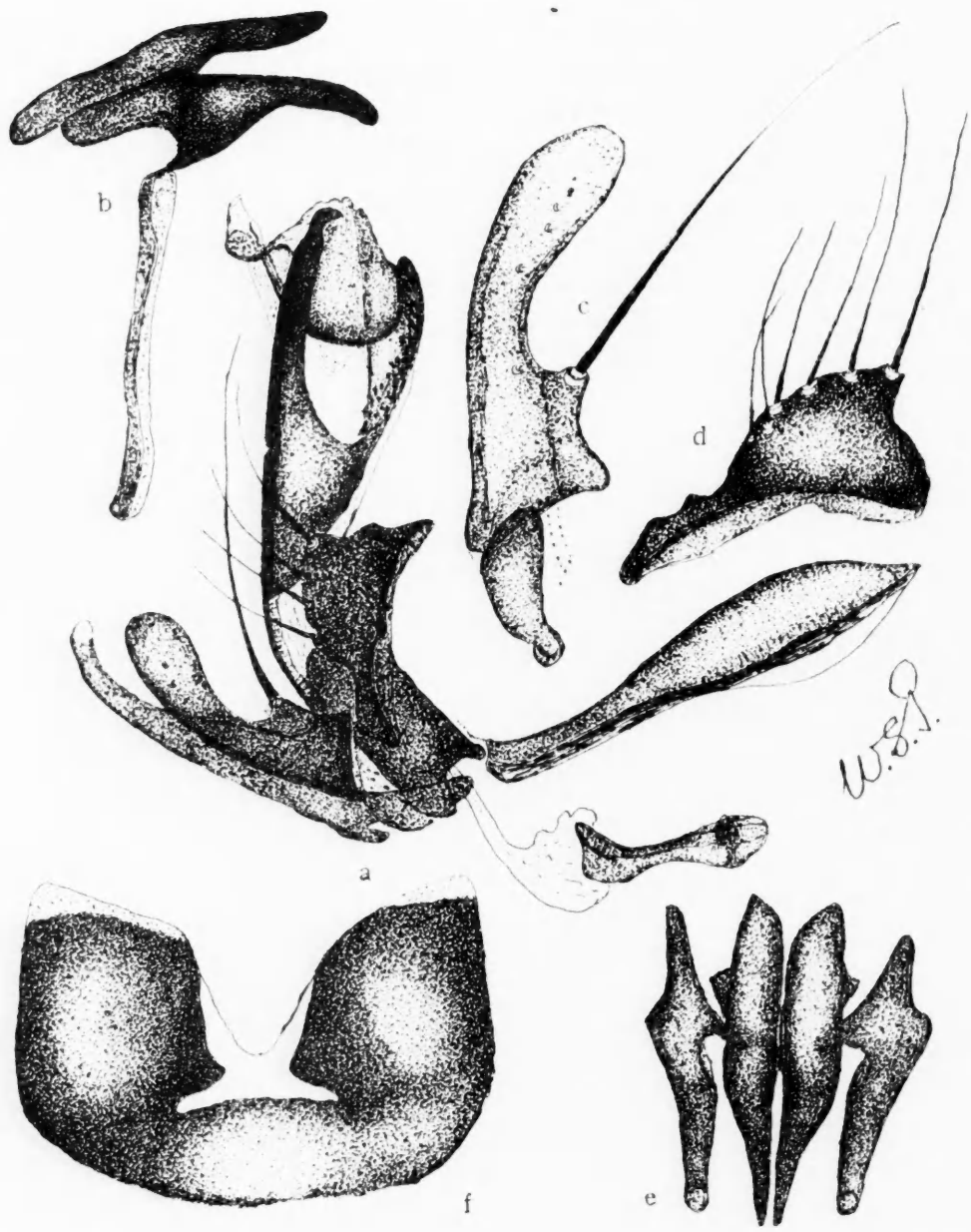

FIG. 3. *a.*—Phallosome and one paramere of *canimicans* in side view ; *b.*—Anal cercus and ninth coxite of same in side view ; *c.*—Right posterior part of paramere ; *d.*—Right anterior part of paramere ; *e.*—Ventral view anal cerci and distal segments of ninth coxites ; *f.*—Fifth sternum of same (Brisbane specimen).

hooked process on each anterior edge of the tenth tergum close to its articulation with the ninth tergo-sternum. I have no doubt that when the female terminalia are studied it will be possible to offer some explanation for these hooks ; the processes offer an easy means of identifying the species. The terminalia will be illustrated and described in due course.

ERYTHROCEPHALA GROUP

Calliphora ochracea Schiner. SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. SCLERITES. Tergum 5 as in fig. 4, *b*. APPENDAGES. *Ninth Coxite*. *Distal Segment*. Ventral view. Fig. 4, *d*. Rather long, broadly expanded,

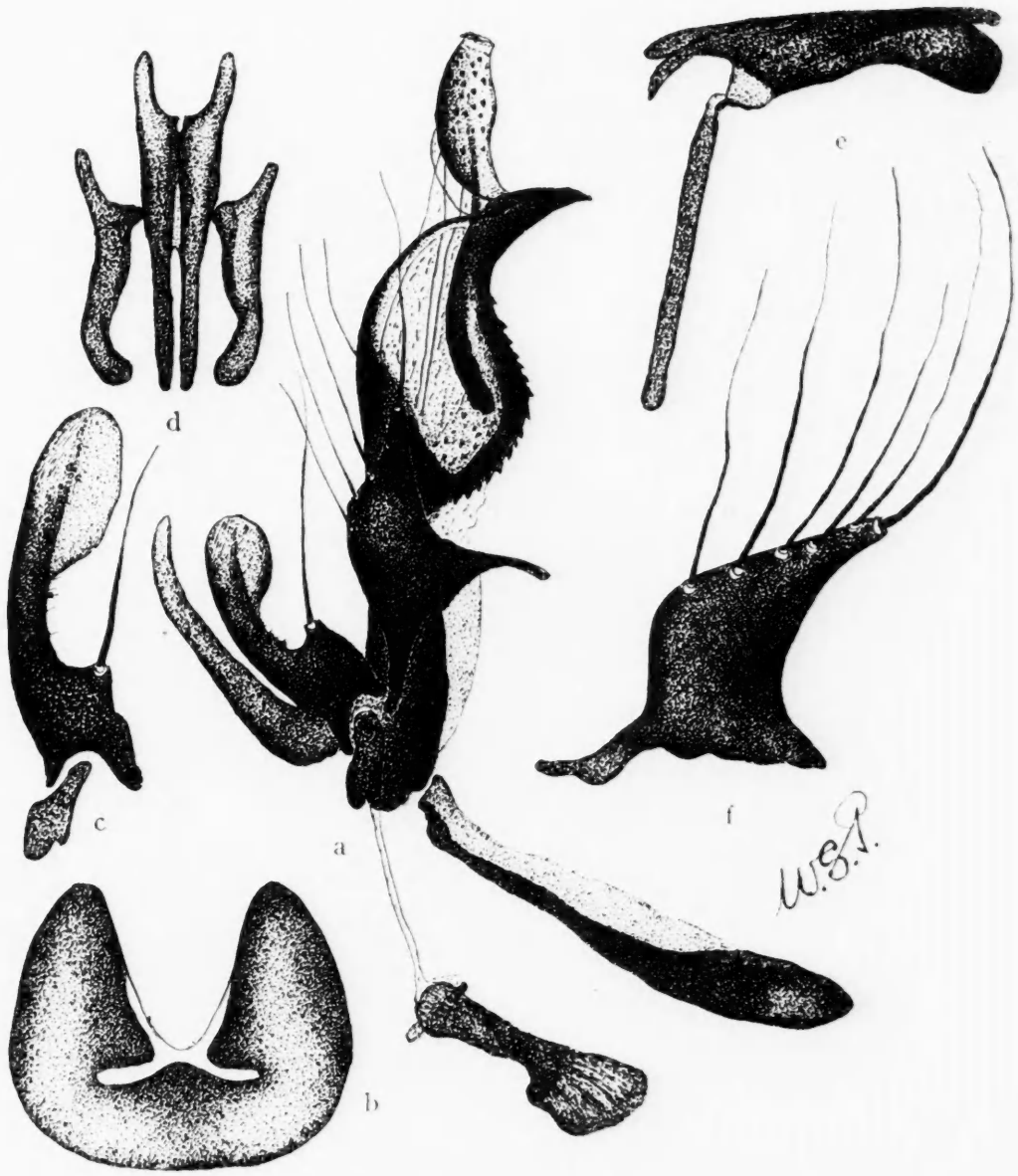

FIG. 4. *a*.—Phallosome and one paramere of *ochracea* in side view; *b*.—Fifth sternum of same; *c*.—Right posterior part of paramere of same; *d*.—Anal cerci and distal segments of ninth coxites of same; *e*.—Lateral view of anal cercus and ninth coxite of same; *f*.—Right anterior paramere of same (Brisbane specimen).

the rounded end turned inward. Lateral view. As in fig. 4, *e*. *Proximal Segment*. Lateral view. A long rather narrow rod joined to its fellow by chitinized membrane. *Anal Cercus*. Ventral view. Fig. 4, *d*. A long pointed rod, the anterior half free. Lateral view. As in fig. 4, *e*. *Phallosome*. Lateral view. Fig. 4, *a*. Structurally similar to that of *erythrocephala*, *vomitoria*, etc. (cf. fig. 4, *a*, with figs. 4, *a*, 6, *a*, 9, *a*, 11, *a*, in this journal, vol. 28, p. 209 et seq.).

The struts slender and ending in a similar expansion, but not free, as in the European and American species of the group ; the end attached to membrane. *Posterior Process of Phallosome.* Lateral view. Fig. 4, *a.* Long and narrow. *Sperm Pump Sclerite.* As in fig. 4, *a. Apodeme of Phallosome.* As in fig. 4, *a. Parameres.* Lateral view. *Anterior Part.* Fig. 4, *a, f.* Very similar to that of *erythrocephala*, with 5-6 hairs (cf. fig. 4, *f*, with fig. 4, *f, g*, in this journal, vol. 28, p. 209). *Posterior Part.* Fig. 4, *a, c.* Also very similar to that of *erythrocephala*, but more expanded distally.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. Fig. 5. The ovipositor is long—almost as long as

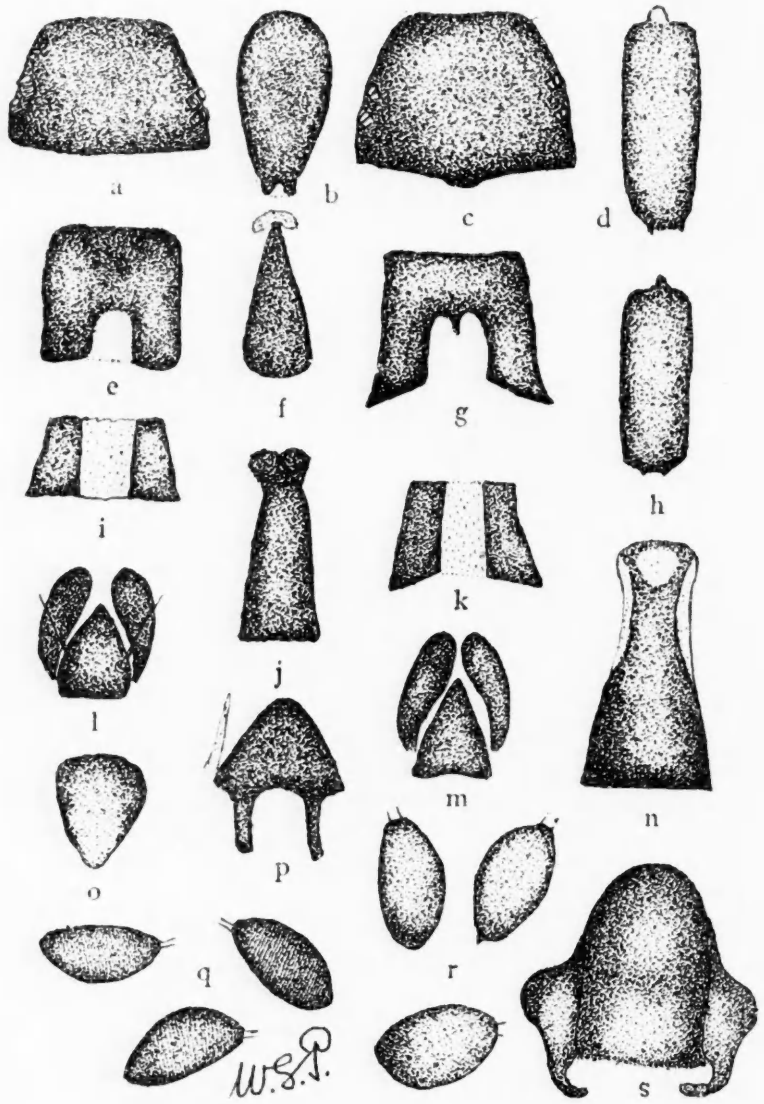

FIG. 5. Sclerites, etc., of ♀ *ochracea* and *vomitoria*. *a.*—Sixth tergum of *ochracea* ; *b.*—Sixth sternum of same ; *c.*—Sixth tergum of *vomitoria* ; *d.*—Sixth sternum of same ; *e.*—Seventh tergum of *ochracea* ; *f.*—Seventh sternum of same ; *g.*—Seventh tergum of *vomitoria* ; *h.*—Seventh sternum of same ; *i.*—Ninth tergum of *ochracea* ; *j.*—Ninth sternum of same ; *k.*—Ninth tergum of *vomitoria* ; *l.*—Tenth tergum and anal cerci of *ochracea* ; *m.*—Tenth tergum and anal cerci of *vomitoria* ; *n.*—Ninth sternum of *vomitoria* ; *o.*—Chitinous plate of uterus of *ochracea* ; *p.*—Tenth sternum of *ochracea* ; *q.*—Spermathecae of *ochracea* ; *r.*—Spermathecae of *vomitoria* ; *s.*—Tenth sternum of *vomitoria* (Brisbane and British specimens respectively).

that of *erythrocephala* (fig. 6) when fully extended The various sclerites and spermathecae are illustrated in fig. 5, as are also those of *vomitoria* (fig. 5) and *erythrocephala* (fig. 6) for comparison. It will be noted that they are all similar to those of the European species. The seventh sternum, perhaps more than any of the other sclerites, offers a good character for identifying the species. That of *vomitoria* (fig. 5, *h*) is long and wide ; that of *erythrocephala* (fig. 6, *c*) narrower ; whereas that of *ochracea* (fig. 5, *f*) is intermediate in size and is more pointed, with a cap-like expansion ; these and other differences can be made out by extending the ovipositor and examining the ventral surface.

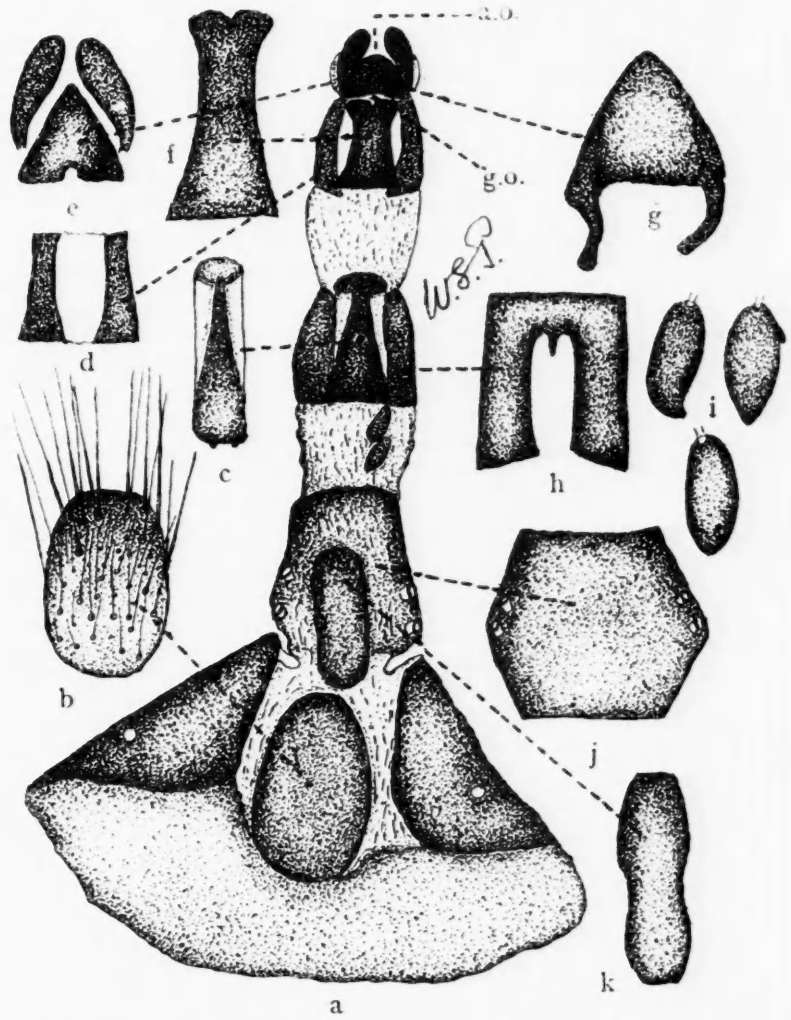

FIG. 6. *a.*—Ventral view of fully extended ovipositor of *erythrocephala* ; *b.*—Fifth sternum of same ; *c.*—Seventh sternum of same ; *d.*—Ninth tergum of same ; *e.*—Tenth tergum and anal cerci of same ; *f.*—Ninth sternum of same ; *g.*—Tenth sternum of same ; *a.o.*—Anal opening ; *g.o.*—Genital opening ; *h.*—Seventh tergum of same ; *i.*—Spermathecae of same ; *j.*—Sixth tergum of same ; *k.*—Sixth sternum of same (British specimens).

As will be shown in subsequent papers, the phallosome of the two other Australian species of the group (*australis* and *stygia*) are structurally similar to that of *ochracea.* And it is interesting to note that the ovipositor is also long and that the sclerites are similar in structure, confirming the relationships shown by the phallosome.

Calliphora grahami Aldrich. SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. This species was described by the late Dr. Aldrich from the mountainous regions of China, Siberia and California. I collected a series from the sacred mountain of Taishan in Shantung, North China. The tenth tergum, anal cerci, ninth coxites, phallosome guide, ninth tergo-sternum and phallosome are illustrated in side view in fig. 7, and the details of the phallosome, etc., in

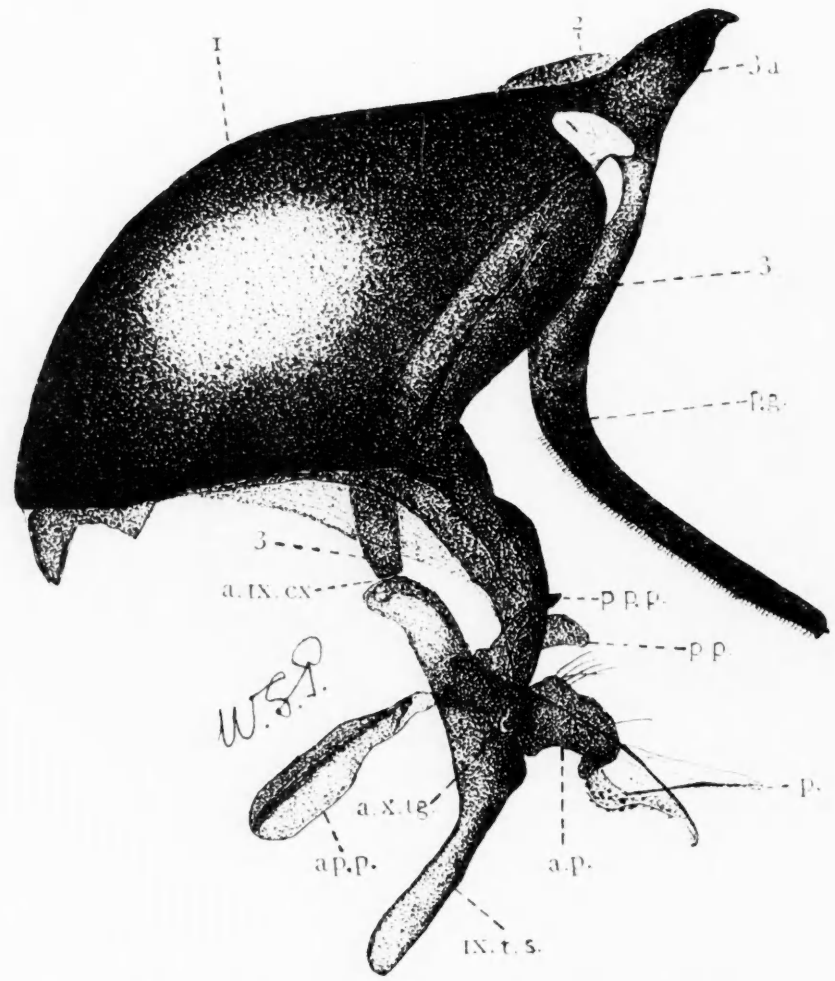

FIG. 7. Terminalia of ♂ *C. grahami* in side view. *a.p.*—Anterior part of paramere ; *a.ix.cx.*—Articulation of ninth tergo-sternum with ninth coxite ; *a.x.tg.*—Articulation of anterior process of tenth tergum in socket of ninth tergo-sternum ; *ap.p*—Apodeme of phallosome ; *p.*—Phallosome ; *p.g.*—Phallosome guide ; *p.p.*—Posterior part of paramere ; *p.p.p.*—Posterior process of phallosome ; 1.—Tenth tergum ; 2.—Anal cerci ; 3.—Proximal segment of ninth coxite ; 3*a*.—Distal segment of same (Chinese specimens).

fig. 8. SCLERITES. Sternum 5 (fig. 8) is very large (drawn to same scale as that of the Australian species in this paper) and has two processes on each side, with a deep emargination between them ; the outer pair is rounded, and the inner pair somewhat quadrilateral in shape; the emargination between the inner pair is deep, to accommodate the phallosome guide. There is a pad of long hairs on the ventral surface of the sternum, as shown in fig. 8. Tergum 6 is well developed, extending laterally. Sternum 6 is long and wide and has a deep long flange with a notch on the dorsal side ; it is attached about the middle of the

dorsal surface of tergum 5. Tergum 10 (fig. 7) is a long sloping plate with a strong dorsal process which articulates by a blunt point in the notch at the side of the ninth tergo-sternum. The ninth tergo-sternum (fig. 8, *b*) is long and narrow, and the posterior processes are fused.

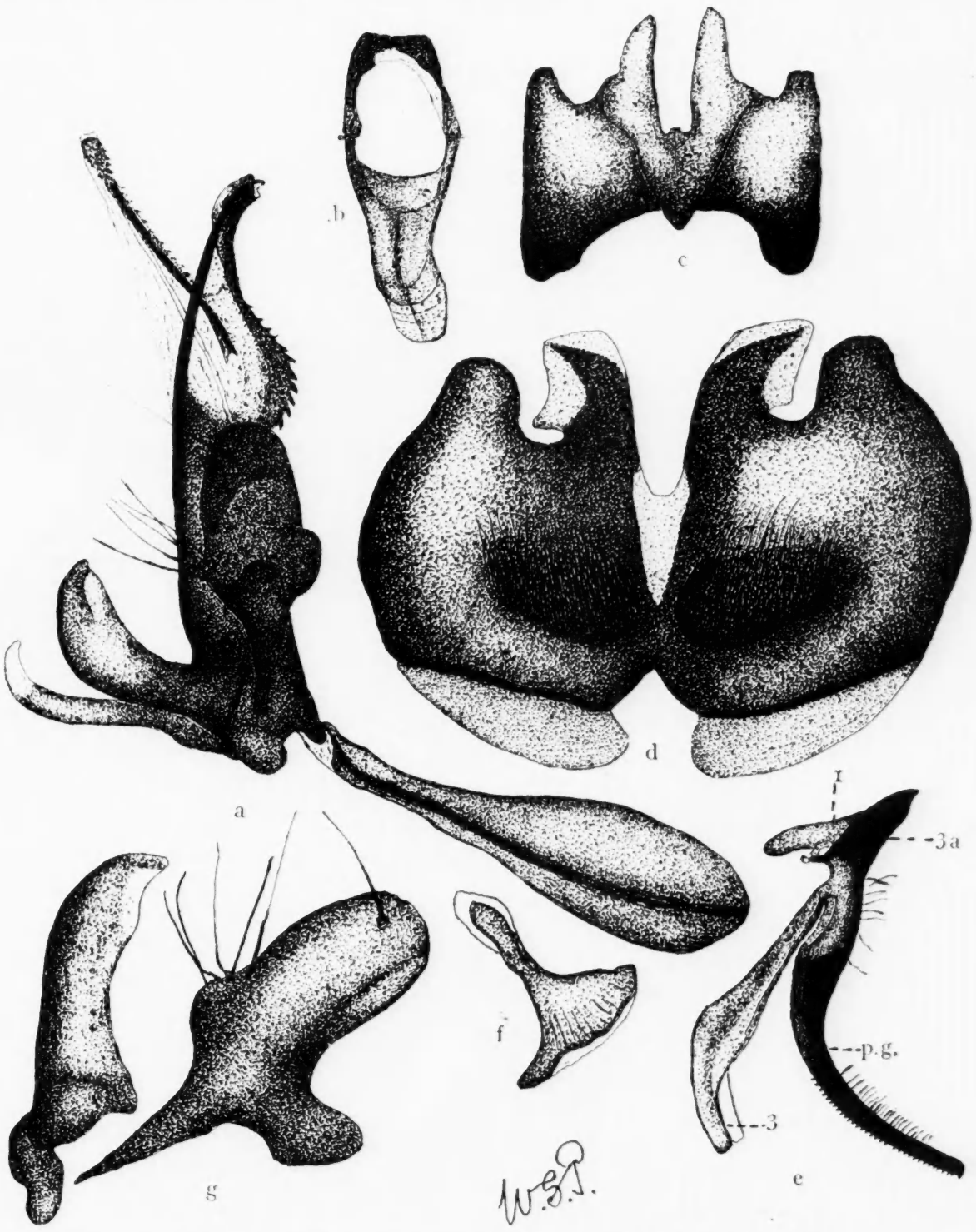

FIG. 8. *a.*—Phallosome and one paramere of *grahami* in side view ; *b.*—Ninth tergo-sternum of same ; *c.*—Ventral view of anal cerci and distal segments of ninth coxites of same ; *d.*—Fifth sternum of same ; *e.*—Anal cercus, ninth coxite and phallosome guide in side view ; *p.g.*—Phallosome guide ; 1.—Anal cercus ; 3.—Proximal segment of ninth coxite ; 3*a.*—Distal segment of same ; *f.*—Sperm pump sclerite of same ; *g.*—Left paramere of same(Chinese specimens).

Appendages. *Ninth Coxite.* Ventral view. *Distal Segment.* Fig. 8, *c.* Short and broad in this view. Lateral view. Figs. 7 ; 8, *e.* In this view it appears short, narrowing to the end, which is pointed and slightly hooked. *Phallosome Guide.* Figs. 7 ; 8. Projecting dorsally from the postero-dorsal end of each distal segment of the ninth coxite, there is a long stout rod, concave anteriorly ; it is slightly hooked at the end, and the posterior convex surface is armed with numerous stiff, short, comb-like hairs ; the two rods are joined medially. *Proximal Segment. Ninth Coxite.* Lateral view. Figs. 7 ; 8. A stout, wide, bent rod curved forwards and slightly expanded at articulation with posterior processes of ninth tergo-sternum. *Anal Cerci.* Ventral view. Fig. 8, *c.* Very small, fused anteriorly to form a median nipple-like process ; posterior end long and free. *Phallosome.* Lateral view. Fig. 8, *a.* Proximal part long ; struts long, expanded at ends, which are excavated and membranous. The membranous anterior spined flaps somewhat reduced and at end expanded into a short chitinous plate which is attached to rods covering them. The distal part of the phallosome consists of a long, chitinous, rod-like plate which extends a considerable distance and is heavily spined. *Posterior Process of Phallosome.* Lateral view. Fig. 8, *a.* Long and curved. *Sperm Pump Sclerite.* As in

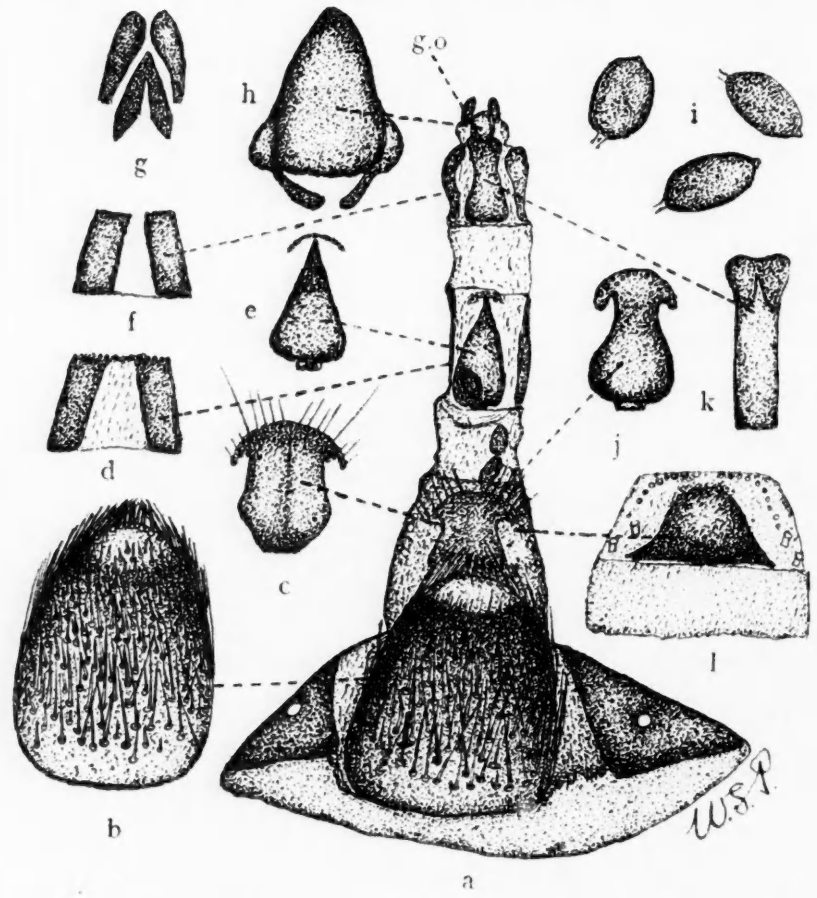

Fig. 9. *a.*—Ventral view of fully extended ovipositor of *grahami* ; *b.*—Fifth sternum of same ; *c.*—Sixth sternum of same ; *d.*—Seventh tergum of same ; *e.*—Seventh sternum of same ; *f.*—Ninth tergum of same ; *g.*—Tenth tergum of same ; *g.o.*—Genital opening ; *h.*—Tenth sternum of same ; *i.*—Spermathecae of same ; *j.*—Sixth sternum of same ; *k.*—Ninth sternum of same ; *l.*—Sixth tergum of same (Chinese specimens).

fig. 8, *f*. *Apodeme of Phallosome*. Lateral view. Fig. 8, *a*. Long and wide. *Parameres*. Lateral view. *Anterior Part*. Fig. 8, *a*, *g*. A broad, rounded plate, with a subapical hair and a group of 5-6 hairs along posterior curved border. *Posterior Part*. Fig. 3, *a*, *g*. A stout upstanding plate without any hairs.

Salient Diagnostic Characters of Female Terminalia. Ovipositor. Segments 6, 7, 9 and 10. The fully extended ovipositor, sclerites and spermathecae are illustrated in fig. 9. The special interest attached to this ovipositor is that the fifth sternum is a very large oval plate armed, especially along the posterior end and margins, with numerous short and long stiff hairs and bristles. When the ovipositor is withdrawn it overlaps the end (genital opening), and it is clear that, in order for the phallosome to enter the genital opening, it would be necessary to lift this plate. For this purpose the long curved rods (phallosome guide, figs. 7 ; 8) have developed on the distal segment of the ninth coxite. In all other respects the ovipositor is like that of *erythrocephala* (cf. fig. 6 with fig. 8), and the phallosome is like that of a species of *Calliphora*. I can, therefore, see no reason for placing this species in a special genus, *Aldrichiella*, as suggested recently by Rohdendorf (1931). It is interesting to note here that *C. auronotata* Macq. is closely related to *grahami*, and that the male has a stout curved hook on each side of tergum 10 near the distal segment of the ninth coxite. When an explanation was sought for these structures, it was found that sternum 6 of the ovipositor is modified in much the same way as sternum 5 is in the female *grahami*. These curious changes in the terminalia of two closely related species

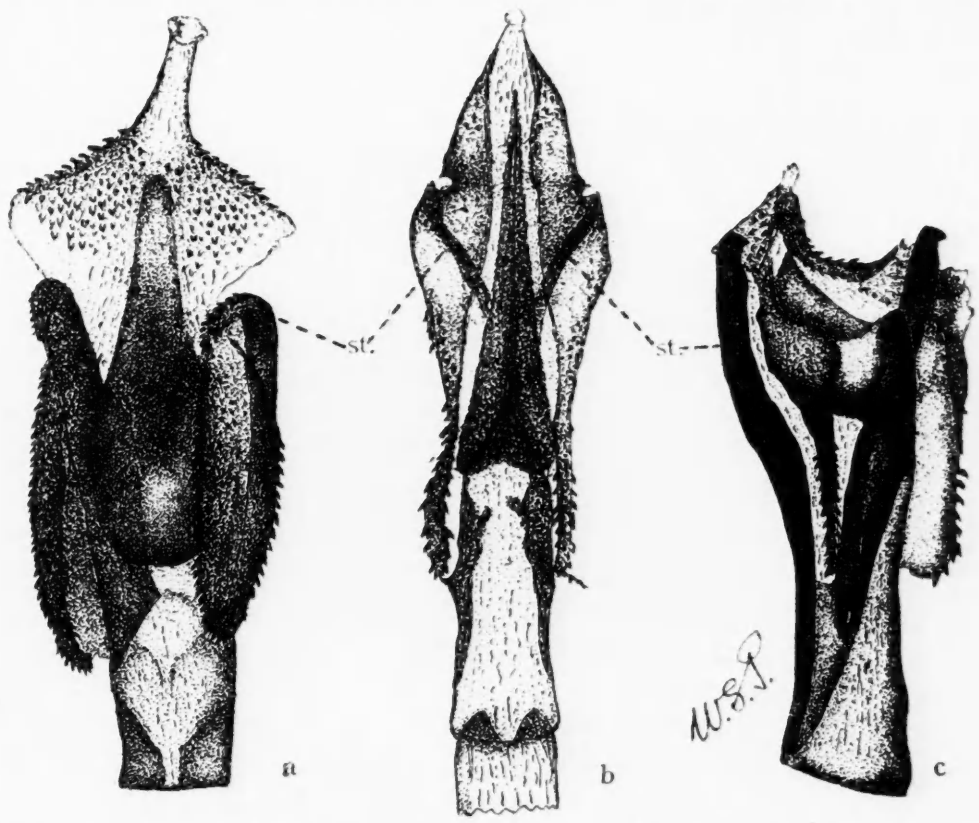

Fig. 10. *a*.—Dorsal view of phallosome of *augur*, showing struts, spined membranous flaps and expanded end ; *b*.—Same of *ochracea* ; *c*.—Same of *canimicans* ; *st*.—struts.

naturally suggests that they have similar breeding habits. Are the larvae parasites of an earth-worm ? Or are they blood-suckers feeding on the blood of some burrowing rodent or on the nestlings of some bird ? The modified sterna would suggest that there is some necessity for protecting the end of the ovipositor during oviposition. These are mere guesses, and I hope that Chinese and Australian observers will investigate the life-history of the species.

It will be noted, then, that in the genus *Calliphora* the phallosome and ovipositor give the clues to the relationships of the species. In fig. 10 is shown the dorsal view of the three types of phallosome ; I believe that that of *augur* represents the primitive form. This type of phallosome has evolved in two directions known at present : the *erythrocephala* type and the *canimicans* type ; the phallosome of *grahami* and *auronotata* exhibits yet another type, and related to it is the species *lilaea* Walker (*ilerda* Walk. ; *latifrons* Hough). Similarly, I believe that the short ovipositor as exhibited by the *augur* group is the ancestral form. This type of ovipositor has also evolved in two directions, and has become either very long, as in the *erythrocephala* group (species of *Musca*, etc., *Hypoderma*, *Gasterophilus*), or even shorter, as in *Auchmeromyia* ; the latter form has become further reduced into the characteristic larvipositor of the Sarcophaginae, Tachininae, *Glossina*, etc.

List of species (with localities) needed to complete this guide :—

Calliphora quadrimaculata Swederus	...	Tasmania.
,, *erythrocephala* Meig.	...	Cosmopolitan.
,, *auronotata* Macq. ...	...	Sydney, N.S.W.
,, *fuscofemorata* Malloch	...	Kuranda, Queensland.
,, *centralis* Malloch ...	...	Upper Lansdowne, N.S.W.
,, *macleayi* Malloch ...	...	Fungella, Queensland.
,, species ? (noted by Malloch)		Botany Bay, N.S.W.
,, *dispar* Macquart ...	...	Uralla, Sydney, etc., N.S.W.
,, *apicalis* Malloch ...	...	Kosciusko, N.S.W.
,, *robusta* Malloch ...	...	,, ,,
,, *xanthocera* Malloch	...	,, ,,
,, *australica* Malloch ...	...	,, (Dec., March) ; Blackheath (Dec.) ; Fish River, Blue Mts., N.S.W.
,, *minor* Malloch ...	...	Townsville, Darwin, Cairns, Queensland.
,, *plebia* Malloch ...	...	Cairns, Queensland.
,, *auriventris* Malloch	...	Fish River, N.S.W.
,, *clarki* Malloch ...	...	Perth, W. Australia.
,, *accepta* Malloch ...	...	Botany Bay, Como, Blue Mts., Sydney, N.S.W. ; Mooroopna, Victoria.
,, *metallica* Malloch ...	...	Woolgoolga, Orange, N.S.W.
,, *assimilis*	...	Marwood, near Mackay, Queensland.

The last 12 species belong to the genus *Onesia*.

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(To be continued)

THE *FUNESTUS* SERIES OF *ANOPHELES* IN SOUTHERN RHODESIA, WITH DESCRIPTION OF A NEW VARIETY

BY

A. M. EVANS

(*Liverpool School of Tropical Medicine*)

AND

H. S. LEESON

(*London School of Hygiene and Tropical Medicine*)

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At the invitation of the Government of Southern Rhodesia, one of us (H.S.L.) recently investigated the breeding places of anopheline mosquitoes in that country. During the tour, extensive investigations were also carried out on *Anopheles funestus* and on the form described as subspecies *leesoni*. Numbers of adults have been bred out from isolated larvae of both these forms, as well as from a third which proves to be a new variety of *funestus*. These bred series, together with numerous batches of eggs obtained from numbered ♀♀, have provided a basis for comparison of the characters of these three forms of the *funestus* series as they occur in Southern Rhodesia.

Anopheles leesoni Evans

Anopheles funestus subsp. *leesoni* Evans (1931)

A. funestus subspecies *leesoni* was described from larvae and pupae which differed in many respects from those of *A. funestus* type form but resembled those of *A. listoni* (*fluviatilis*) in most of these characters. In 1933, De Meillon, who had discovered *leesoni* in the Transvaal in December, 1931, was able to describe the adult characters from bred material and to give a more complete account of the species. Since the publication of his paper, this author has discovered a marked difference in the structure of the pharynx and egg (1934) between *leesoni* and typical *funestus*, and informs one of us that he finds that the ♂ terminalia of *leesoni* differ in certain respects from that of *funestus* type form, but resemble that of *fluviatilis* in these characters. Examination of our material shows that Southern Rhodesian *leesoni* does not differ from Transvaal material in these respects. We are much indebted to Dr. De Meillon for permission to refer to the pharyngeal character and for the use of his drawings.

The number of important structural differences between *leesoni* and *funestus* type form makes it evident that the two forms are specifically distinct. The marked difference is, however, somewhat diminished by the presence in Southern Rhodesia of a very common new variety of *funestus* which is described

below. This form resembles, or approaches, *leesoni* in certain respects, but has much more in common with the type form of *funestus* ; it also possesses a few characters peculiar to itself.*

The resemblance between *A. leesoni* and *fluviatilis* in the adult stage is extremely close, but marked differences in the larval and egg stages have recently been observed. In view of the fact that Christophers (1933) is unable to record any marked morphological distinctions between *A. fluviatilis* and the oriental *minimus*, and states that to distinguish between these forms ' may be difficult if the palpal ornamentation is ambiguous,' it is clear that some of the oriental species in the series are also very closely related. The striking difference in size and shape of the tergal plates (see below) distinguishes the larva of *fluviatilis* from that of *leesoni* more definitely than from that of *minimus*. Thus *leesoni* is as clearly differentiated from *fluviatilis* as are certain other oriental species of the series from each other.

ADULT. ♀. Closely resembling *A. fluviatilis* and *A. funestus*.

Head. Frontal tuft well developed. Palpi, as in *funestus*, usually with the subapical pale band narrow, the dark area distal to it about 3 or 4 times its length, but sometimes with the subapical pale band considerably broader, more than half the length of the dark band.

Pharynx (fig. 1, D—G). De Meillon has discovered that the hair-like processes of the post-armature (pharyngeal) ridges are very short in *leesoni*, in marked contrast to *funestus* in which they are long. We have found this character also present in Southern Rhodesian ♀♀ of *leesoni*, the processes of the ridges being very short, the length of the longest being less than half the greatest width of one of the ridges. In 2 ♀ *fluviatilis* from Kasauli, presented to the Liverpool School of Tropical Medicine by Colonel Sir S. Rickard Christophers, one of us has recently found that the processes of the ridges are also relatively short.

Thorax and *Abdomen*. No differences from *A. funestus* have been found.

Wings. No constant difference from *A. funestus*. As in that species, the length of the main costal spots is subject to a good deal of variation, and the sector spot may be incomplete. Base of costa normally all dark, but a well-marked pale interruption present in one bred ♀. Third vein almost always with middle 1/3 pale and, most commonly, mainly pale with small dark areas at or near the ends (fig. 3, B) ; exceptionally, the vein was entirely dark (3 wings, bred specimens) or with minute pale spot ; the pale area usually occupies the middle portion of the vein (each of 12 ♀♀ determined by eggs laid, and in 23 out of 26 bred specimens). Fifth vein, stem with a few pale scales at the base succeeded by a dark area to about 1/3 of its length, followed by a longer pale area which reaches almost to the bifurcation where the scales are dark ; upper branch with a small pale area at the cross-vein ; lower branch with a pale area, variable in length, beyond the bifurcation. Sixth vein as in *funestus*.

* See also paper, p. 69, infra, describing yet another form.

Wing length. Limits among 8 caught ♀♀
determined by eggs laid :—3·0–3·2 mm.
16 bred ♀♀ :—2·6–3·2 mm.
13 „ ♂♂ :—2·4–3·5 mm.

Legs. As in *A. funestus.*

ADULT. ♂ (fig. 4, b). Palpi with pale spot or band at base of club, in addition to the pale areas at apex and middle, which are normally present in *funestus.* This was noted in the original material by Evans (1931), and was definitely established as a character of *leesoni* by De Meillon (1933), whose illustrations we reproduce.

Hypopygium (fig. 1, A—C). The structure closely resembles that of *fluviatilis* as described by Christophers (1933). Harpago with club variable in shape. External accessory hair about 2/3 length of apical, usually single, but 2 present in 2 specimens examined. A very delicate internal hair, somewhat shorter than external, sometimes present. Phallosome with about 3 or 4 leaflets at each side, the largest being definitely smaller than in *funestus*, the length being about 33–36μ in *leesoni* and about 44–49μ in *funestus* (by A.M.E.'s standard of measurement).

PUPA (fig. 5, A). Differing from that of *A. funestus* type form in the following features of the abdominal chaetotaxy, the most striking of which were noted by Evans (1931) and De Meillon (1933).

Paddle. Accessory hair normally with 3 or more branches.

Spine A. V—VII, more than half length of succeeding segment ; IV, about half length of succeeding segment.

Bristle B. VII, with about 5 branches ; VI, 5 or 6 ; V, 5 ; IV, about 11–14.

Bristle C. V—VII, simple and longer than succeeding segments, usually about one and a half times their length ; IV, about 10–16 branches.

Hair 4. VII, about 4–5 branches ; VI, 6–9 ; V, 8–11 ; IV, 10–14. On IV—VII this hair arises from a point directly anterior to bristle C.

LARVA (figs. 6–8). Differing from that of *A. funestus* type form mainly as follows :—

Head. Anterior half of fronto-clypeus typically with a pair of longitudinal dark bands, which are usually continuous with the post-frontal transverse band but are sometimes reduced to isolated areas or are even absent ; De Meillon (1933) notes that the fronto-clypeus may occasionally be almost entirely dark. Posterior clypeal hairs usually placed further behind inner than in *funestus*, overlapping the bases of these by only a small fraction of their own length. Posterior frontal hairs divided into about 3–10 branches, and usually arising nearer the middle line than in *funestus.*

Thorax. Palmate hair very similar to that of *funestus*, but with 16–21 leaflets which may be slightly wider than in that species.

Abdomen. Integument of ventral surface without the belts of well-developed

spicules characteristic of *funestus*. De Meillon, who first referred to this difference (1933), has also pointed out (in litt.) that the spicules along the postero-lateral borders of segment X are inconspicuous and much smaller in *leesoni* than in *funestus* (fig. 8, D). We are much indebted to this author for permission to refer to this character and to another mentioned below. Main tergal plates shallower than on corresponding segments in *funestus*. Shape of plates on V—VII characteristic, the general outline roughly oblong ; anterior margin convex, sometimes more strongly so in middle ; posterior margin almost always slightly concave or almost straight, at least in its middle 1/3 ; if convex, only slightly so ; lateral border relatively long (in comparison with that of *fluviatilis*). Posterior border characteristically with a narrow, uniform rim of darker chitinization. Median accessory plate included in main plate, but not the *posterior paired plates* (fig. 7, C, *p.a.*), which are usually present about half way between the main plate and posterior border of the segment. Median plate of scoop of spiracular apparatus (fig. 8, E) with lateral arms usually shorter and more slender than in *funestus* and, as found by De Meillon, the distance between the tips of the arms considerably less than in that species ; the average figure in *leesoni* is about 0·05–0·055 mm. and in *funestus* about 0·085–0·09 mm. In studying this character it has been recognized that the arms are often foreshortened in mounted larvae and only cases where they were flat, or almost so, have been included in the measurement. Hair ' O ' on III—VII usually situated outside the postero-lateral angles of the main tergal plates ; details as follows : on III, variable in size, 1–2 (rarely 3) ; IV, variable in size, 1–3 (4) ; V, small, 1–2 (3) ; VI, small or minute, 1 (2) ; VII, minute, 1–2.

Certain differences from the larva of *fluviatilis* were pointed out by one of us (A.M.E., 1931), the most important being the smaller average number of branches of hair ' O,' which also appears to be more variable in size than in *fluviatilis* and is usually much smaller on segments V—VII. More recently, we have observed a much more striking difference from *fluviatilis* as described and illustrated by Puri (1931). Through the kindness of Dr. Puri, we have been able to compare the larvae of *leesoni* with those of authentic *fluviatilis*, and Dr. De Meillon has recently informed one of us that two larvae he received from Colonel Sinton differed markedly from those of *leesoni* in the character in question. Main tergal plate much larger than that of *fluviatilis* ; this also differs from that of *leesoni* in having the posterior border usually definitely convex posteriorly and the lateral border relatively short, so that the shape is not at all oblong as in *leesoni*. In one specimen from Karnal, sent by Dr. Puri, the plates were very shallow and not convex behind, but the median accessory plate was not included in the main plate on III—VI. One of us (A.M.E.) has consulted Dr. Puri about the shape of the arms of the median plate of the scoop in *A. fluviatilis*. He kindly informs us that they are very variable in size and shape in that species and may be similar to those of *leesoni* as seen in sketches sent to him ; he notes the effect of pressure on the scoop in influencing the apparent

shape and size of the arms in mounted larvae. In the two larvae sent by Dr. Puri and in one other *fluviatilis*, the arms are short, but the plate itself is considerably broader at this level than in any larvae of *leesoni* examined. Possibly, therefore, this latter character may afford a further distinction between the larvae of the two forms.

Egg (fig. 10). Batches of eggs obtained from caught ♀♀ were preserved on lint kept damp with a solution of formalin (5 parts), glycerine (2 parts) in distilled water (93 parts). They agreed with Transvaal specimens recently described by De Meillon (1934) in the characters of the *frill* which is unbroken and extends the full length of the egg, and of the *floats*, which are separated above by almost the full width of the egg. The following details were also observed :— when the egg is viewed in lateral aspect, the middle portion of the under-surface appears only slightly convex.

*Convex surface** ornamented with polygonal network at each end, but in the middle region unornamented, i.e., covered with very small bosses of practically uniform size (fig. 10, C).

Examination of the eggs, with the 1/6 objective by transmitted light, in the above-mentioned fluid, shows that the reticulated appearance near the poles is produced by polygonal areas of the small bosses enclosed by ' walls ' of much larger bosses, as shown in fig. 10, B. This is probably the condition which De Meillon refers to as ' irregularly marked with patches of black pigment in certain lights.'

The ornamentation in *costalis* and many species of subgenus *Anopheles* is similarly produced and probably also that in *cinereus*, *theileri* and *longipalpis*, according to De Meillon's recent description and figures.

The egg differs from that of *fluviatilis* as described by Christophers and Barraud (1931) in that the frill is well developed throughout the length of the egg and is, at most, slightly narrower in the middle than at the ends. The presence of a polygonal network at each end of the convex surface is a more important, though less obvious, difference.

Bionomics—See page 41.

Distribution in Southern Rhodesia. *A. leesoni* was obtained from the following districts:—Salisbury (4,800 ft.), Shamva (3,600 ft.), December, 1933; Sinoia (3,870 ft.), Trelawney (4,000–5,000 ft.), January, 1934 ; Salisbury, Ndanga (3,500 ft.), Mashoko (3,000 ft.), February, 1934 ; Umtali (3,670 ft.), Nyaderi (3,000–4,000 ft.), Penhalonga (3,000–4,000 ft.), Old Umtali (3,000–4,000 ft.), March, 1934 ; Salisbury, April, 1934.

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*' Convex surface ' is here used instead of ' lower surface ' of Christophers and Barraud (1931), which is often inappropriate when applied to the whole surface outside the level of the frill or frills.

Anopheles funestus Giles type form

The characters distinguishing this species from its oriental allies have been described by Christophers and Puri (1931), King (1932) and Evans (1930). Most of those differentiating *funestus* from *fluviatilis* also separate it from *A. leesoni* and have been noted by Evans (1931) and more fully by De Meillon (1933). In these characters and also in those of the pharynx discovered by De Meillon and of the larvae found by that author and the present writers, as well as in the egg characters described by Christophers and Barraud (1931), Gibbins (1933) and De Meillon (1934), South African specimens do not differ materially from those found in more northern parts of the Ethiopian region.

The following is a summary of the main characters in which the type form of this species differ from *A. leesoni*.

ADULT. ♀. *Pharynx* (fig. 2, D, E). As found by De Meillon, the hair-like processes of the post-armature ridges are long, the longest being longer than the greatest diameter of the ridges and sometimes approaching twice this width.

Wing. Basal 1/4 of costa sometimes with a small pale interruption, but more commonly entirely dark. Third vein most frequently either entirely dark (except for minute pale spots at the ends) or with a small pale area beyond the middle ; pale area not usually more than 1/3 the length of the vein and never as extensive as in fig. 3, B. Fifth vein with pale areas on stem very variable in extent, upper branch with a pale spot at the cross-vein ; the spot occasionally reduced to 1 or 2 pale scales.

Wing length. Measurements of extremes among caught ♀♀ from various localities :—2·4 mm. (Freetown)—3·3 mm. (Belgian Congo).

ADULT. ♂. *Palpi* (fig. 4, a). Club without pale scales at its base.

Terminalia (fig. 2, A—C). Harpago with external accessory hair less than half the length of the apical, usually about 2/5 its length, and appearing stouter than in *leesoni* owing to its shortness. Inner accessory hair usually absent. Phallosome with about 5 or 6 pairs of leaflets, the largest being about 44–49μ (by A.M.E.'s standard of measurement).

PUPA (fig. 5, C). As noted by Evans (1931) and Senevet (1932), the chaetotaxy of the pupa exhibits considerable variation even amongst specimens from the same region, and we have found certain differences between material from Sierra Leone, Uganda and Southern Rhodesia, but the numbers of pelts examined are too small to be of definite significance. The limits in the number of branches given below are based mainly on material from these regions.

Paddle. Accessory hair simple or bifid.

Spine A. V—VII, less than half length of succeeding segments ; IV, about 1/3—1/2 that on VII.

Bristle B. VII, with about 2–5 branches ; V and VI, 2–6 ; IV, 4–7.

Bristle C. V—VII, about as long as succeeding segments. VII, simple or bifid; VI, simple or with 2–3 (sometimes 4) branches; V, 2—3-branched or simple; IV, 3–8.

Hair 4. VII, 1–4; VI, 2–4; IV and V, 3–5. On IV—VII this hair arises anterior to a point between the bases of *B* and *C* (midway between them in Southern Rhodesian pelts examined).

LARVA (figs. 6–8). Fronto-clypeus with a transverse dark band extending across anterior half and well separated from the post-median band. Posterior clypeal hairs overlapping base of inner by about 1/2–2/3 their own length; as noted by King (1932), *A. funestus* differs from its oriental allies in this respect. Posterior frontal hairs usually simple, sometimes bifid (De Meillon, 1933, records one case with 4 branches).

Thorax. Palmate hair with about 13–17 very narrow leaflets.

Abdomen. Integument of ventral surface with conspicuous belts of spicules, most strongly developed on IV—VI; this character was noted by King (1932) and by De Meillon (1933), and had also been observed by one of us. Spicules arising from membrane at each side of posterior wall of segment X (fig. 8, D) unusually large and well developed. This character was recently pointed out to one of the writers by Dr. B. De Meillon, who has kindly permitted us to refer to it. Main tergal plates very large and deep, the depth on IV—VII being considerably more than half that of the segments. Shape of plates rather variable but usually somewhat rectangular with rounded posterior angles; posterior margin convex, sometimes only slightly so. Median and paired accessory plates on IV—VII normally included in the main plate, near the posterior border of which they are usually recognizable (fig. 7, E, F). Median plate of scoop of spiracular apparatus with short lateral arms projecting towards the spiracles; distance between tips of arms averaging about ·085–·09.

EGG (fig. 9, D). *Frills* present at each end of upper surface, entirely separate from each other and not extending beyond inner edge of floats. Each frill very narrow or rudimentary and incomplete or open. *Floats* approaching each other on upper surface, the distance between them being not more, and sometimes much less, than 1/3 the width of the egg. *Convex surface* ornamented with relatively very large bosses. The size of the bosses is shown in fig. 9, D, which is a camera lucida drawing of part of the surface of an egg examined in a fluid mountant. When viewed by direct reflected light, the bosses appear as dark areas separated by a white network. The outline of the under surface was observed in Nigerian eggs preserved in formalin. It resembled the outline shown in Gibbins's (1933) figure, being only slightly more convex than in *leesoni*. In De Meillon's figure for Transvaal material the outline is as convex as in var. *confusus* (see below).

DISTRIBUTION IN SOUTHERN RHODESIA. *A. funestus* type form was obtained from the following places:—Shamva (3,600 ft.), November, 1933; Sinoia (3,870 ft.), January, 1934; Ndanga (3,500 ft.), February, 1934; Nyaderi (3,000–4,000 ft.), March, 1934; Salisbury (4,800 ft.), March and April, 1934.

OTHER RECORDS. Transvaal, De Meillon (1933); Northern Rhodesia, 1 larva out of a collection taken in 1932 and kindly lent by Sir Malcolm Watson;

Kenya, 1 larva, received from Mr. C. B. Symes, has been determined as this species.

Anopheles funestus var. **confusus** var. nov.

This variety differs from the type form of *funestus* in certain characters of the larva and egg, as well as in the length of the processes of the post-armature ridges in the ♀. In certain of these respects, the variety resembles or approaches *leesoni*. The markings of the ♂ palps are variable, and may be either as in that species or as in *funestus* type form. The name *confusus* was proposed independently by both writers.

ADULT. ♀. *Pharyngeal armature* (fig. 2, F). Hair-like processes of post-armature ridges shorter than in *funestus* type form, the longest usually being about equal in length to the width of a ridge. In one ♀ identified by the eggs laid, the processes appeared to be somewhat shorter than this, but exact measurements could not be made with this mount.

Wing (fig. 3, A). Base of costa with or without a pale interruption. Third vein entirely dark or with pale area, the longest observed being about half the length of the vein ; pale area, when present, usually situated distal to middle point of vein. This condition was constant among 12 bred specimens in which this vein showed a pale area ; in the other 5 bred specimens the vein was dark. Upper branch of fifth vein, unlike that of type form, usually entirely dark, but small pale area present in one ♀ ; lower branch of fifth vein with extended pale area.

Wing length. Limits among 6 caught ♀♀
determined by eggs laid :—3·1–3·5 mm.
5 bred ♀♀ :—2·7–3·5 mm.
5 ,, ♂♂ :—2·8–3·5 mm.

ADULT. ♂. *Palps* with or without a pale spot at base of club.

Terminalia. Usually as in *funestus* type form. In one bred ♂, the external accessory hair of the harpago was slightly more than half the apical and more slender than in typical *funestus* ; the leaflets in this specimen were shorter than in *funestus*.

PUPA (fig. 5, B). Very similar to that of type form.

Paddle. Accessory hair simple or bifid.

Spine A. Very short on segment IV, longest on VII, but only rarely attains a length equal to half that of succeeding segment.

Bristle B. VI and VII, with about 3 branches ; V, 4 or 5 ; IV, about 4 or 5 (7 in one case).

Bristle C. About equal in length to succeeding segments V—VIII, simple but sometimes bifid ; IV, about 4 branches.

Hair 4. V—VII, 2 or 3 branches ; IV, about 4. The position of this hair is more lateral than in *leesoni*.

LARVA (figs. 6, 7). *Head.* Fronto-clypeus with anterior transverse band,

sometimes well developed, but often narrow or ill-defined, or, rarely, absent. Posterior clypeal hairs overlapping bases of inner by about half their own length. Posterior frontal hairs simple or sometimes bifid.

Thorax. Palmate hairs with 13–19 leaflets.

Abdomen. As in the type form, integument of ventral surface of IV—VI with well-marked belts of spicules. Main tergal plates much shallower than in *funestus* type form and very similar in shape to those of *leesoni*, the posterior border concave or straight and outline roughly oblong, but no definite darker rim posteriorly as in *leesoni*. Median accessory plate included in main plate, but paired accessory plates separate and situated in the membrane some distance behind posterior border of main plate. Hair 'O' on III—VII usually situated well on tergal plates, but sometimes very near the edge, and in one specimen they were just off the edge of the plates on III and V at least ; the hair, in most specimens, very coarse and dark on III—VII, and thus much more conspicuous than in *funestus* ; simple or bifid on IV—VI, usually simple on VII.

Egg (fig. 9, A—C). No variation was observed among seven batches of eggs obtained from caught ♀♀. In the character of the *frills* and *floats* the egg resembles that of *funestus* type form, from which it differs as follows :—*Convex surface* covered with bosses which are very much smaller than those of the type form ; the relative size of the bosses in the type and the present variety is shown in fig. C and D. As indicated in the drawings, the bosses in both species are very irregular in size. No polygonal network present.

In the character of the abdominal tergal plates, this form resembles *A. manganyanus* Banks, as described by King (1932), but it is clear from the characters of the ♀ that the resemblance does not indicate relationship.

Distribution in Southern Rhodesia. Type ♂ with larval and pupal pelts, Umtali (3,670 ft.), March 6th, 1934 ; type ♀ with pelts, Sinoia (3,870 ft.), January 11th, 1934 ; paratypes 2 ♂♂ and 2 ♀♀ with pelts, Sinoia (3,870 ft.), and Umtali (3,670 ft.), 1934 ; all material taken and bred out by one of the writers (H. S. Leeson). The variety was also taken in the following localities :— Ndanga (3,500 ft.), February, 1934 ; Nyaderi (3,000–4,000 ft.), March, 1934.

Types in British Museum ; paratypes in London School of Hygiene and Tropical Medicine and in Liverpool School of Tropical Medicine.

Bionomics of the three Southern Rhodesian forms of the *funestus* series

Adult Habits. *Occurrence in houses.* Adults of the three forms have been taken in houses, but this point was not specially studied, and so it is not possible to say in what proportion the different forms frequent human habitations.

Outdoor haunts. Along the banks of streams adults may be found among the grass and weeds, but particularly in crevices in the earth and in cavities in the banks. At river crossings, known as ' drifts ' in Southern Rhodesia, adults hide away in the daytime underneath the stones which are usually piled

Table indicating main differences between three African anophelines of the *funestus* series

	A. funestus type form	**A. funestus** var. **confusus**	**A. leesoni**
FEMALE			
Pharynx : processes on post-armature ridges	long	shorter than *funestus*	very short
MALE			
Palpi : pale scales at base of club	absent	present or absent	present
Phallosome : leaflets... ...	44–49μ	as *funestus*	33–36μ
PUPA			
Paddle : access. hair ...	simple or bifid		3 or more branches
Spine A, IV : length ...	very short		$\frac{1}{2}$ segment
„ V—VII : length ...	less than $\frac{1}{2}$ segment		more than $\frac{1}{2}$ segment
Bristle B, IV : branching...	4–7		11–14
„ *C*, „ : „ ...	3–8		10–16
„ „ V—VII : „ ...	simple or 2–3 branched		simple
„ „ „ : length...	about equal to 1 segment		longer than 1 segment, often $1\frac{1}{2}$ × segment
Hair 4, IV : position ...	more lateral		anterior to *C*
„ „ : branching ...	3–5		10–14
LARVA			
Fronto-clypeal markings ...	anterior transverse band		paired longitudinal stripes or areas
Post-frontal hair	simple or bifid		3 or more branches
Tergal plates : size	large, deep	smaller, shallow	
„ „ : post. margin	convex	concave or straight	
Post. paired access. plates ...	on main plate	off main plate	
EGG			
Frill	at ends only, open		full egg-length, closed
Floats : distance between ...	less than 1/3 egg-width		full egg-width
Convexity of under side ...	slight	great	very slight
Bosses : arrangement ...	evenly distributed		polygonal areas of small ones enclosed in walls of large ones (near poles)
„ : size...	large	smaller than *funestus*	see previous line

up to enable road traffic to cross the river. In times of flood, of course, these places are washed out ; but soon after, when the water has subsided, the adults frequent these places again. Another outdoor haunt is the disused quarry or gravel-pit ; here the adults will be found in similar situations, under stones

and in crevices in the sides of the pit. All these places are protected from the sun and wind and are consequently dark, damp and warm.

Breeding Places. Larvae of all three forms are found in the same types of water: the edges of slowly moving streams among the roots of the grass and weeds which provide the necessary shade and protection. The water is usually clear. They have been taken in swamps, that is, in permanent collections of water on the ' veld ' with aquatic vegetation ; in these there is an almost imperceptible movement of the water.

Seasonal Variation in Abundance and Haunts: Effect of Altitude. In the wet season (November to April) adults can be found in all the usual situations, both indoors and out, and larvae in most streams—*leesoni* perhaps a little earlier than *funestus* and *confusus*. In the dry months (June to September) the numbers of larvae and adults are very much reduced. In fact, it is with the greatest difficulty that any larvae can be obtained at all ; but a few adults may be seen. They have been collected from beneath the rocks and boulders in the beds of rivers near Shamva at about 3,000 ft. altitude. At 1,500 ft., adults of *funestus* were taken inside a hut in July, 1928. It is suggested that at such altitudes, where the temperature remains comparatively high throughout the year, the mosquitoes are able to maintain themselves in their various stages, whereas at higher altitudes either they are killed off or they hide away until the coming of the warmer weather.

Acknowledgement. The authors are greatly indebted to Professor P. A. Buxton for the helpful interest which he has taken in the investigation, and for his most valuable suggestions.

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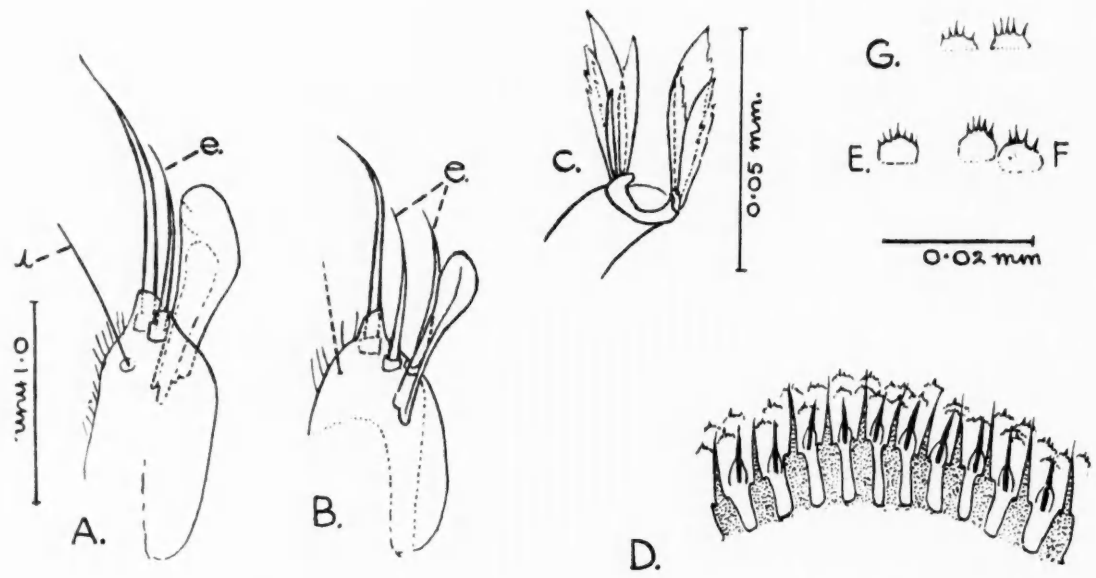

FIG. 1. *Anopheles leesoni* Evans : A. and B.—Harpago showing variation ; C.—Leaflets of phallosome ; D.—Pharyngeal armature ; E.-G.—Post-armature ridges to larger scale ; e.—external ; i.—internal accessory hairs. (D. copied diagrammatically from a drawing by Dr. B. De. Meillon ; the rest drawn by A.M.E.)

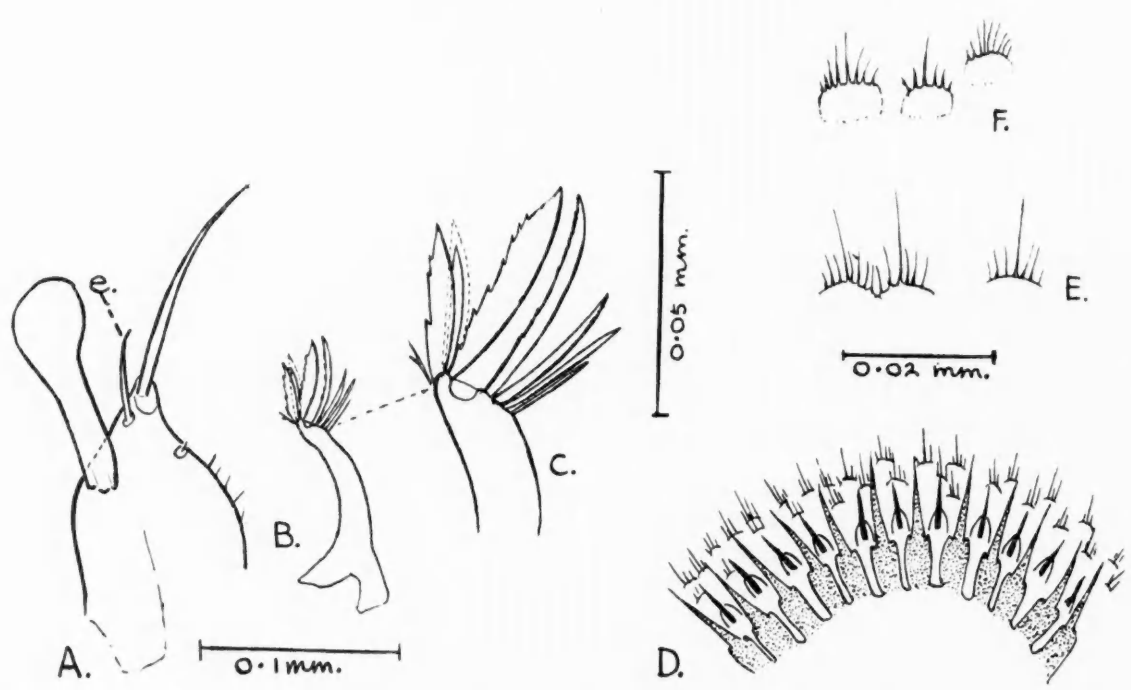

FIG. 2. *Anopheles funestus* Giles type form : A.—Harpago of Freetown ♂ ; B.—Phallosome ; C.—Leaflets to larger scale ; D.—Pharyngeal armature ; E.—Three post-armature ridges to larger scale. *A. funestus* var. *confusus* var.n. : F.—Three post-armature ridges ; e.—external accessory hair. (D. copied diagrammatically from a drawing by Dr. B. De Meillon ; the rest by A.M.E.)

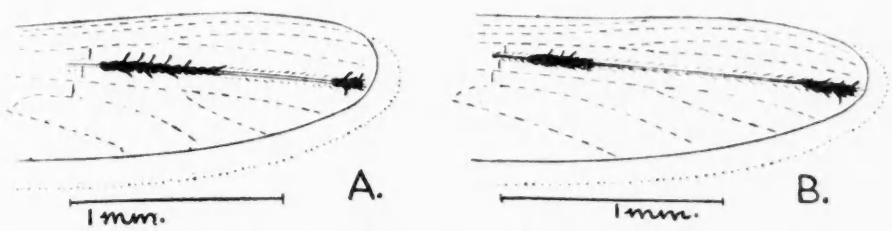

FIG. 3. Outline of part of wing of ♂ to show usual condition of third vein. A.—*A. funestus* var. *confusus*; B.—*A. leesoni*. (Drawn by H.S.L.)

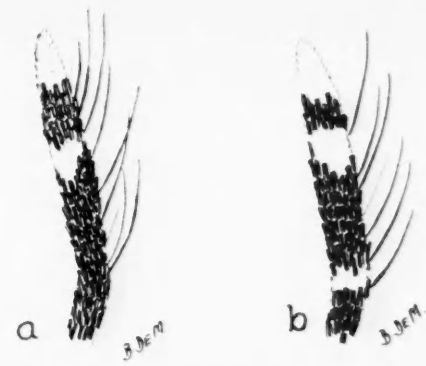

FIG. 4. Apex of ♂ palp to show base of club. a.—*A. funestus* type form; b.—*A. leesoni*. (After De Meillon.)

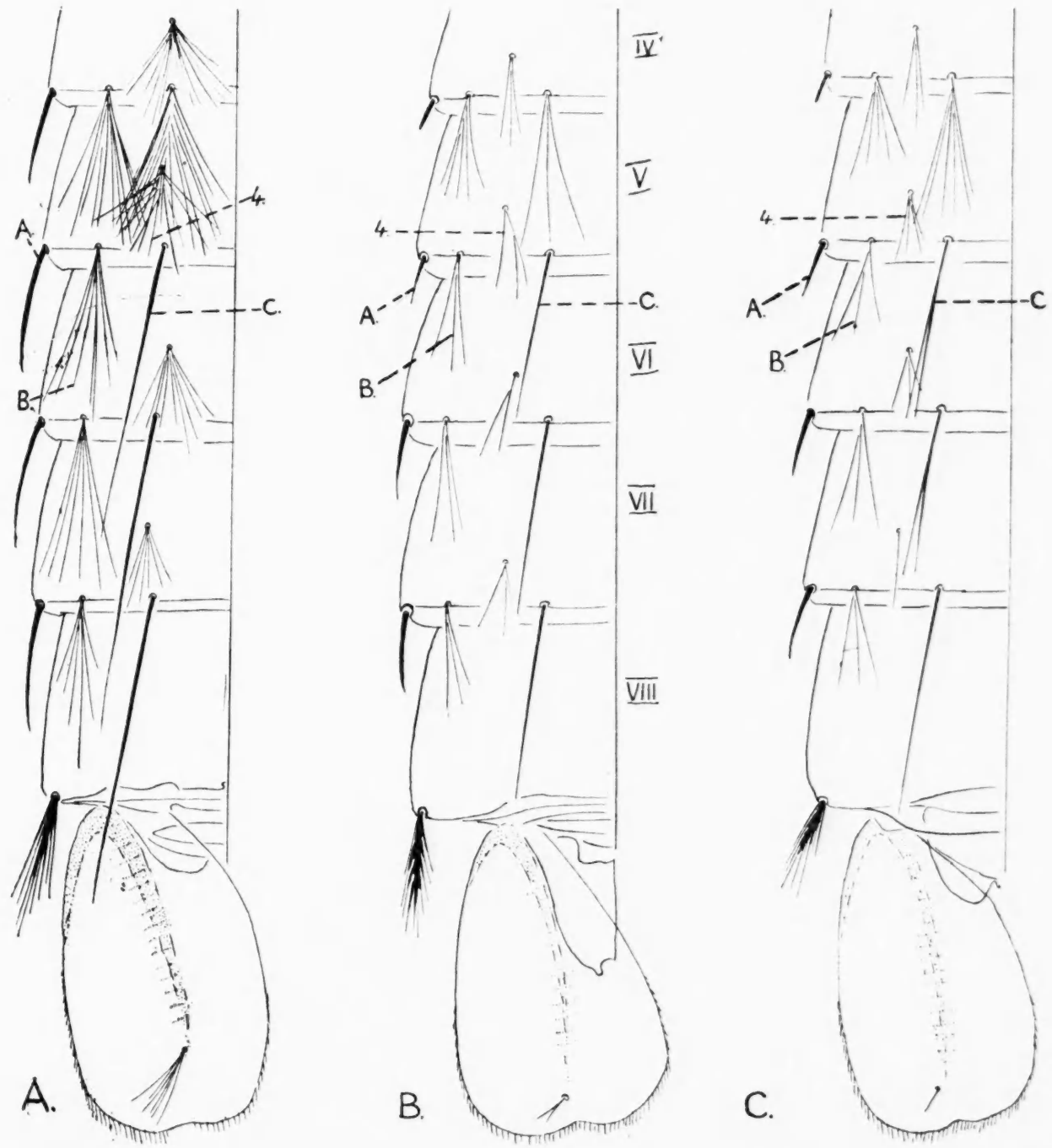

FIG. 5. Pupa : last five segments of abdomen, dorsal aspect, semi-diagrammatic, to show the number of branches of the more important bristles and hairs. The accessory paddle hair, though ventral, is readily seen ; apical paddle bristle not shown. A.—*Anopheles leesoni* ; B.—*A. funestus* var. *confusus* ; C.—*A. funestus* type form. Spine *A*, bristles *B* and *C* and hair **4** are lettered in each case on segment V. (Drawn by H.S.L.)

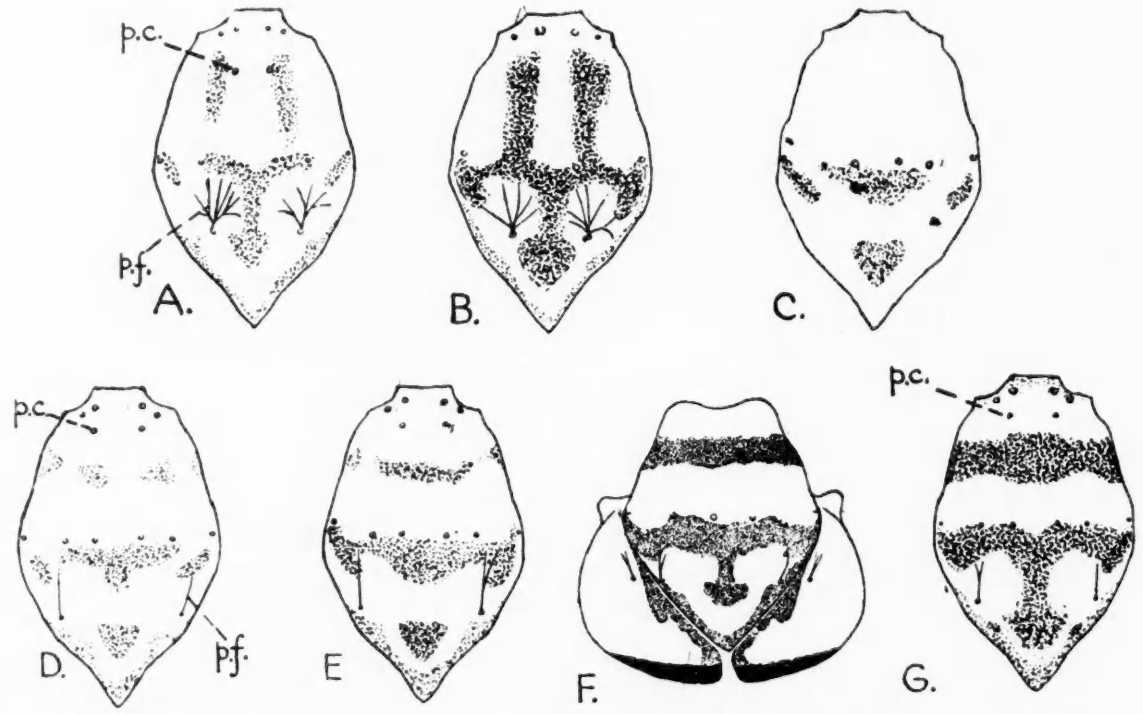

FIG. 6. Larva : fronto-clypeus (F.—whole head) to show chiefly the markings on anterior half and the post-frontal hairs. *A. leesoni* : A. and B.—typical, C.—atypical ; *A. funestus* type form : F. and G. ; *A. funestus* var. *confusus* : D. E. and F.—typical, C.—atypical. p.c.—position of posterior clypeal hair ; p.f.—post-frontal hair. (F. after De Meillon ; the rest by A. M. E.)

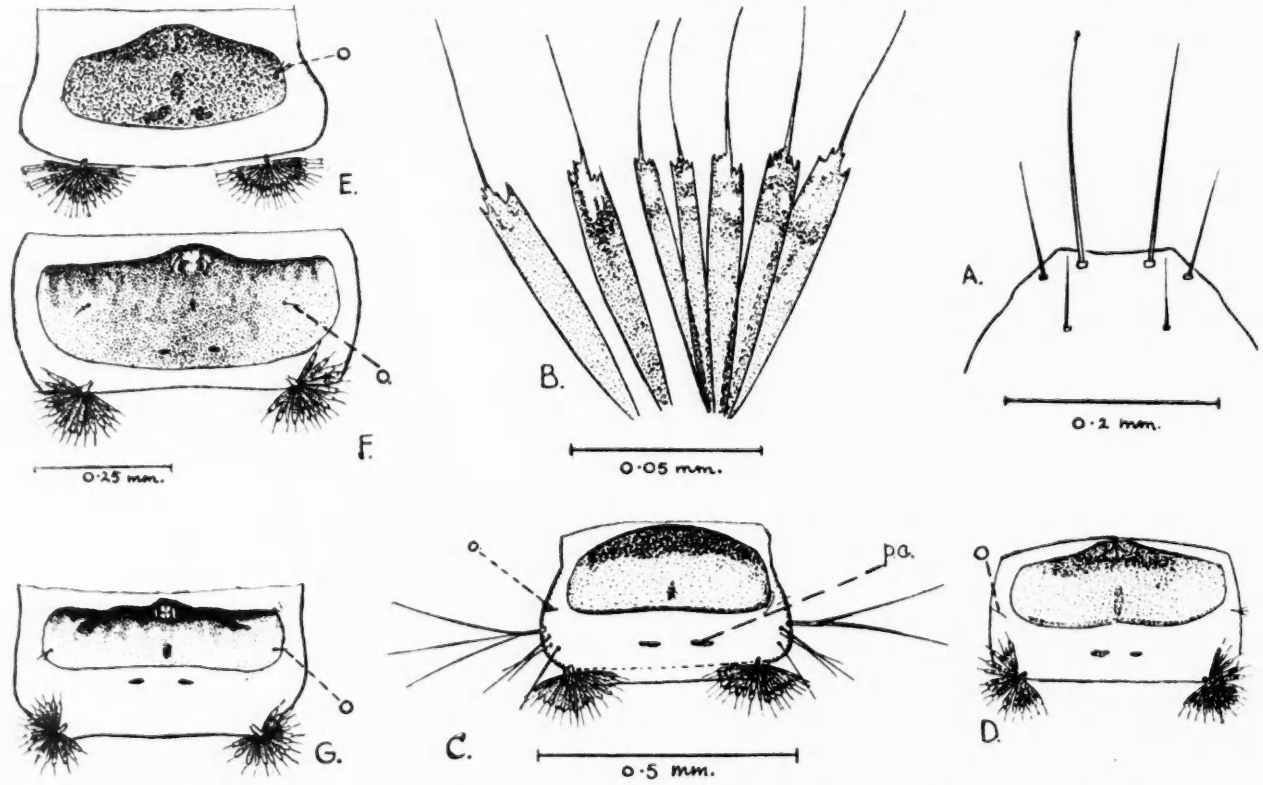

FIG. 7. Larval details. *A. leesoni* : A.—Clypeal hairs ; B.—Leaflets of palmate hair of V (from three specimens) ; C.—Abdominal segment V of larva mounted without pressure ; D.—The same of larval pelt (tergal plate flat). *A. funestus* type form : E. and F.—Segment V (E. less flattened than F.). *A. funestus* var. *confusus* : G.—Segment V flattened ; C.-E. to same scale ; F. and G. to same scale ; o.—hair ' o ' ; p.a.—paired accessory tergal plates. (F. and G. drawn by H.S.L. ; the rest by A.M.E.)

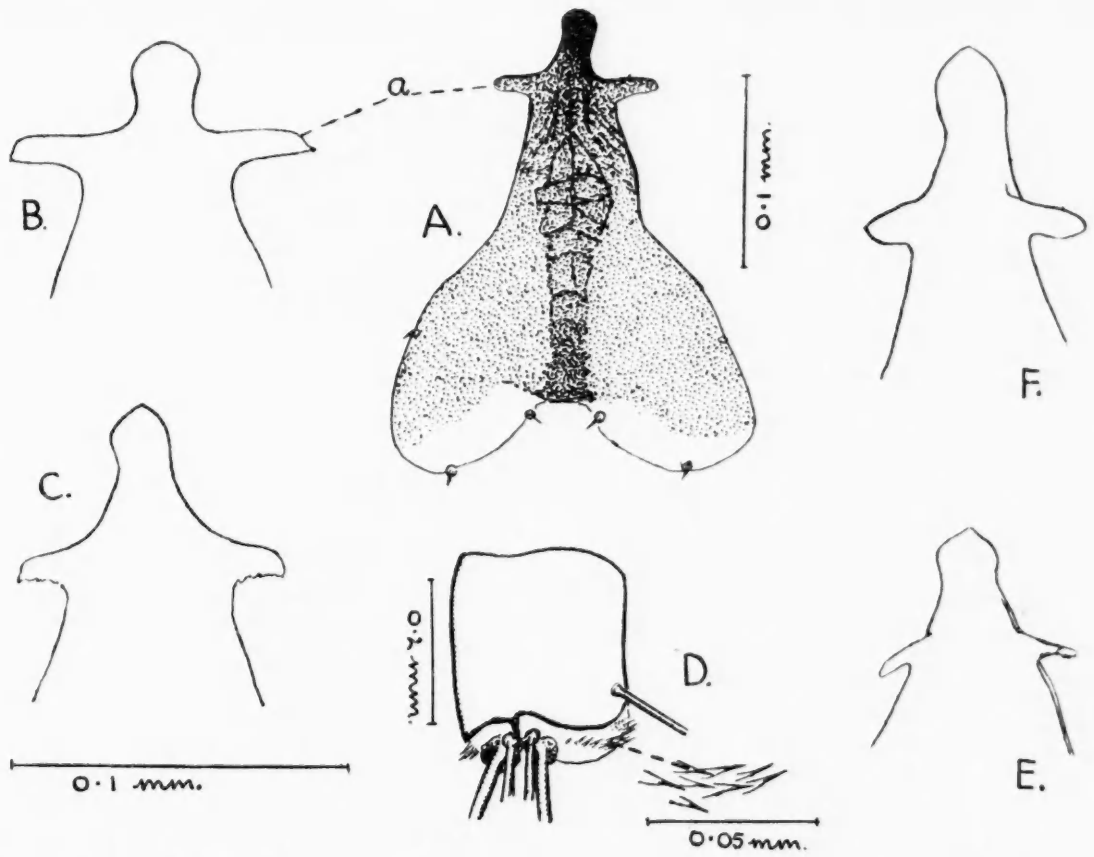

FIG. 8. Larval details. *Anopheles funestus* type form : A.—Median plate of scoop of spiracular apparatus ; B.—Anterior portion of plate to larger scale, typical ; C.—The same, less typical ; D.—Segment X, dorso-lateral aspect, spicules shown to larger scale. *A. leesoni* : E.—Anterior portion of plate, typical ; F.—The same, atypical. a.—lateral arms. (F. after a drawing by Dr. B. De Meillon ; the rest by A.M.E.)

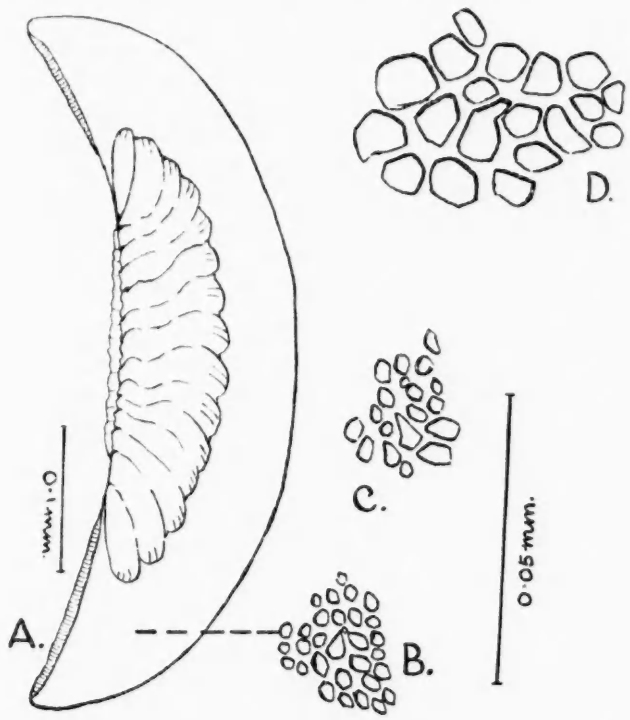

FIG. 9. *A. funestus* var. *confusus* : A.—Egg, lateral aspect ; B. and C.—Bosses on convex surface from two eggs, as seen by transmitted light. *A. funestus* type form : D.—Bosses on convex surface to same scale as B. and C. (A. drawn by H.S.L.; B.-D. by A.M.E.)

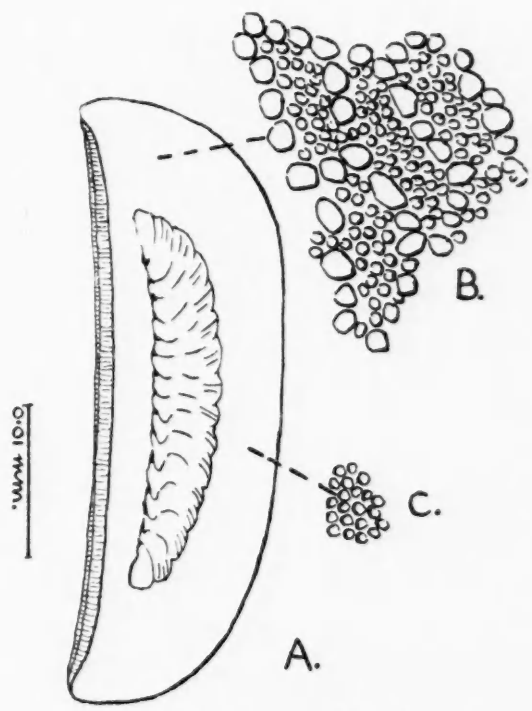

FIG. 10. *A. leesoni* : A.—Egg, lateral aspect ; B.—Portion of convex surface towards pole ; C.—From middle of surface as seen by transmitted light. (A. drawn by H.S.L.; B. and C. by A.M.E.)

THE INTERPRETATION OF THE INCUBATION PERIOD OF THE VIRUS OF YELLOW FEVER IN THE MOSQUITO (*AËDES AEGYPTI*)

BY

ANDREW WATSON SELLARDS

(*From the Department of Tropical Medicine, Harvard Medical School, Boston, Mass.*)

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Of the diseases transmitted by arthropods there is a small group in which the etiological organism is unknown and is readily filterable. Yellow fever is a classical example of this group ; it illustrates the difficulty of obtaining exact information concerning the behaviour of the causative virus in the usual vector, *Aëdes aegypti*. Such phrases as ' the infection of mosquitoes ' and the ' extrinsic incubation period ' indicate the generally accepted view that the virus multiplies in its mosquito host. Valuable evidence concerning this view has been supplied by Dr. Davis, an experienced investigator who unfortunately died recently at Bahia, Brazil.

Davis, Frobisher and Lloyd (1933) have reported an extensive experimental study concerning the question of the multiplication of the virus of yellow fever in the mosquito. Their procedure consisted in grinding infected mosquitoes and titrating the virus content of the resulting suspension by injecting serial dilutions into monkeys. Mosquitoes were selected for titration at three periods, namely, (1) immediately after the infective blood meal, (2) during the incubation period in the mosquito, and (3) at varying intervals after the mosquitoes were able to transmit the virus. These authors have discussed the difficulties inherent in titration experiments of this nature. Their data indicate that there is first a sharp fall in the virus content of the mosquitoes and then probably a gradual rise, which, however, fails to reach the original level found immediately after the infective feeding. In freshly fed insects, the titre was extremely high, a dilution of 1 to 1,000,000,000 producing fatal infections in two experiments. After an interval of 4 to 7 days the virus demonstrable by titration was only 0·1 per cent. to 1 per cent. of the amount found immediately after the infective feeding. Subsequently there was a 50-fold rise in titre in two series of experiments (e.g., from 1–1,000,000 to 1–50,000,000). In a third experiment the titre after the initial fall rose to 1 to 100,000,000, but this represented only a 10-fold increase.

The authors present an interpretation of these data which is important. The initial fall in titre is ascribed in part to a loss of virus by defecation and also to its probable invasion of the cells where it might be less easily freed in the procedure of grinding the mosquitoes. The data indicative of a subsequent

increase in titre are discussed in some detail, considerable emphasis being placed on the conclusive evidence that the amount of virus found during the incubation period never surpassed the quantity originally ingested. They reach the conclusion that there is apparently no propagative nor cyclical change of the virus in the mosquito and that the incubation period represents merely the time required for the virus to migrate to the salivary glands. According to their conception, a marked contrast exists between yellow fever as an example of a filterable virus, *sensu restricto*, and the Rickettsiae which multiply freely in their arthropod host. The mode of transmission of yellow fever is presented by Davis and his collaborators as a type hitherto unknown, since it is neither a biological not a simple mechanical procedure. It appears that promptness of action is regarded as a prerequisite of mechanical transmission. The term ' delayed mechanical ' is proposed by them as a suitable designation to indicate the mode of transmission in yellow fever. This disease, they feel, does not conform to any of the modes of transmission in the following useful classification proposed by Huff (1931) :—

	Multiplication of organism in vector	No multiplication of organism in vector
Cyclical change of organism in vector	I. Cyclo-propagative Example : Malaria by mosquitoes	II. Cyclo-developmental Example : Filaria by mosquitoes
No cyclical change of organism in vector	III. Propagative Typhus by lice	IV. Mechanical Typhoid by flies

The views of Davis and his associates raise several points for consideration in regard to the interpretation of their experimental data and with respect to the general conceptions of the usual behaviour of various parasites in their arthropod hosts. It is of interest that the initial loss in virus was in no case fully regained during the subsequent period of incubation. However, this finding must not be confused with the essential question of whether multiplication of the virus occurs after the initial fall in titre.

Concentration of Virus. In the first place, the infective feedings were given under artificial conditions. Fulminating infections of yellow fever in the monkey probably result in a much higher content of virus in the blood than ordinarily occurs in nature in human patients. If the mosquitoes had been fed minimal amounts of virus, it remains an open question whether the subsequent increase in titre would have exceeded the original level.

Susceptibility of Mosquitoes. After the ingestion of massive quantities of virus by the mosquitoes, the sudden fall in titre is quite in keeping with the behaviour of parasites which are known to multiply freely in their arthropod host. The situation can be made clearer by a consideration of some of the protozoa where the course of the infection in insects can be followed with ease. When the flea ingests the rat trypanosome a considerable number of the organisms degenerate, whereas others penetrate the cells and multiply (Minchin and Thomson, 1915). The observations of Huff (1934) in malaria of birds also afford a clear-cut illustration. With a suitable species of mosquito many individual insects are found to be refractory. But even in the individual mosquitoes known to be susceptible, many of the ingested malarial parasites die. In the case of yellow fever it would theoretically be necessary to know whether the early reduction in titre during the extrinsic incubation period could be accounted for by the presence of considerable numbers of refractory mosquitoes. This is not probable. The subject of a refractory state to yellow fever in individual mosquitoes (*Aëdes aegypti*) has received little attention. In the absence of direct investigation, experience shows that this species is very easily infected. An analysis of the work of the United States army board in Cuba indicated that, in feeding experiments on human patients, about half of the mosquitoes became infected (Gay and Sellards, 1927). With fulminating infections in monkeys it would seem that the proportion is much higher in view of the ease with which infections are obtained with single mosquitoes selected at random from an infected lot.

Effect of Temperature. Temperature has a striking effect on the incubation time of the virus of yellow fever in the mosquito. Marchoux and Simond (1906) noted in all of their experiments that the virus failed to develop in mosquitoes kept at about 20° C. Hindle (1930) found that the incubation period may be indefinitely prolonged at low temperatures (10° to 15° C.). On the other hand, the writer (1930) noted the opposite effect at a high temperature. Five mosquitoes, after an infective feeding, were kept for 2 days at 37° C.; after an additional 4 days at 28° C., they fed on a monkey and produced a fatal attack of yellow fever. Fortunately Davis (1932) has carried out a series of observations at varying temperatures. The incubation time showed a well-marked prolongation as the temperature was lowered, ranging from 4 days at 37° to 18 days at 21° C. Infectivity did not develop after 30 days in mosquitoes kept at 18°, though the virus remained alive and active. This shortening of the incubation at higher temperatures was interpreted at that time as indicating more rapid multiplication of the virus in the mosquito. The writer is in full accord with this former opinion of Davis, in preference to his subsequent view of migration of the virus.

Dissemination of the Virus. Early dissemination of the virus of yellow fever in the mosquito has been reported by Davis and Shannon (1930). Mosquitoes were given an infective feeding and were then tested a few (2 to 6) days later

before their bites had become infective. Injections into monkeys of the anatomical divisions of either the head, the thorax, or the abdomen produced yellow fever. Davis and his collaborators have assumed that the virus must of necessity reach the salivary glands in order for the bite of the mosquito to be infective. It is well to remember that convincing experimental evidence for this interpretation is difficult to obtain and is not yet complete. A consideration of the possible modes of migration of the virus might help to clarify the situation. If one hesitates to accept propagation as the important factor, it becomes difficult to find a tangible explanation. Conceivably the circulatory system might aid in the distribution of the virus, even though the rather meagre evidence indicates that the body-fluid of a mosquito is not infective. If distribution of the virus by the circulatory system is of importance, one would not expect moderate changes in temperatures to produce such marked differences in the incubation periods.

In order to maintain the position that the virus merely migrates in the mosquito, it is necessary to explain its apparent increase after the initial fall and to determine why a higher temperature should greatly accelerate the dissemination of the virus. It appears advisable to me that yellow fever should be retained for the present in the group of arthropod-borne diseases in which the mechanism is one of simple propagation of the virus. The crucial test of this view might be obtained by attempting the serial passage of the virus directly from mosquito to mosquito by feeding normal ones upon ground suspensions of others that have been infected. Marchoux and Simond (1906) recorded the failure of normal mosquitoes (*Aëdes aegypti*) to acquire infection from the cadavers of mosquitoes containing the virus, but their observations were designed to simulate conditions that might occur in nature.

Arthropod Origin. Of the many parasites harboured by insects and other arthropods, only a small proportion are pathogenic for vertebrates. In the course of the evolution of the arthropod transmission of disease it is assumed that some of these parasites have acquired pathogenicity for vertebrates and that they have subsequently lost their ability to maintain themselves solely in arthropods. This conception has been developed especially in connection with some of the flagellates. A filterable virus such as yellow fever does not lend itself easily to such considerations. However, the evidence that this virus presumably multiplies in the mosquito is consistent with the view that yellow fever may have originated in mosquitoes.

SUMMARY

An interesting observation has been presented by Davis, Frobisher and Lloyd (1933). When mosquitoes (*Aëdes aegypti*) ingested massive quantities of the virus of yellow fever there was a prompt and extreme loss of virus followed by a gradual increase, but the initial loss was not fully recovered. The authors

conclude that the incubation period in the mosquito is required, not for the multiplication of the virus, but for its migration to the salivary glands.

The writer prefers a different interpretation of these data. There are well-known illustrations among the protozoa where, upon ingestion by a suitable arthropod host, many of the parasites die and others establish themselves and multiply. The initial loss of the virus of yellow fever in the mosquito is entirely consistent with its subsequent multiplication as an essential factor for successful transmission. Under similar conditions of temperature, the incubation time of this virus is of the same order in either its vertebrate or its invertebrate host (e.g., 4 days at 37° C. in the mosquito). However, the period in the mosquito is progressively prolonged as the temperature is lowered (e.g., 18 days at 21° C.). A plausible explanation of this fact is that the virus grows more readily at the higher temperature. In the search for the causative organisms of yellow fever, several interesting lines of approach are available if one assumes that mosquitoes can be infected with this virus. It seems to me that such experimental studies deserve to be continued, and that the data of Davis and his associates offer encouraging evidence that the virus multiplies in its insect host.

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THE INFECTION AND IMMUNIZATION OF MICE BY INTRAPERITONEAL AND SUBCUTANEOUS INJECTION OF THE VIRUS OF YELLOW FEVER.*

BY

ANDREW WATSON SELLARDS

(*From the Department of Tropical Medicine, Harvard Medical School, Boston, Mass.*)

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Interest in the susceptibility of mice to yellow fever has centred chiefly in the production by Theiler (1930) of encephalitis by intracerebral inoculation. The effects of extraneural injections have given some additional information concerning the behaviour of this disease in mice, especially with reference to immunity and to inapparent infection. Professor Charles Nicolle (1912) first presented evidence of the occurrence of inapparent infection in the course of his investigations in typhus fever, and Nicolle (1933) has recently reviewed the subject in connection with various infections. This conception has had far-reaching importance in the study of infectious diseases.

Inapparent Infection. Mice that had been inoculated subcutaneously or intraperitoneally were subsequently tested for inapparent infection chiefly by three procedures—namely, (1) by inoculation of their blood and other tissues intracerebrally into normal mice ; (2) by observations for demonstrating active or passive immunity ; (3) by the intracerebral injection of starch paste to facilitate the localization of virus in the brain.

MATERIALS AND METHODS

Virus of Yellow Fever. The French strain of yellow fever was used exclusively, employing both the neurotropic and the classical virus. The infectious mouse brain used in the following experiments refers only to the neurotropic strain (125th to 210th passages in mice) after the loss of its ability to produce typical necrosis of the liver. This strain is definitely *neurocytotropic*, but for convenience it is usually known simply as *neurotropic* virus. The serum of infected monkeys (*Macacus rhesus*) has been used as the source of classical virus. In the absence of exact information, the term ' classical ' is chosen to designate the virus of yellow fever as it ordinarily occurs in nature. The word 'viscerotropic,' though frequently used for this purpose, is quite inappropriate. This designation might connote the idea of localization of the virus in the various viscera. Unfortunately the site of multiplication and the distribution of the classical virus is not clearly understood. There remains an element of vagueness concerning the tropism of the viscera for the classical strain. The conception of viscerotropism tends to confuse an essential feature of some of the virus diseases. Tropism, for the protection of its significance, is applicable to types of cells, but not to organs nor to viscera. Furthermore, the writer prefers, till more complete information is available, to use the term ' virus ' conservatively in a general sense, rather than in the restricted meaning of an obligately intracellular parasite.

Source of Mice. In some laboratories, difficulty has been encountered in obtaining white mice that are susceptible even to well-established neurotropic strains of yellow fever upon intracerebral inoculation. On the other hand, Laigret (1933) noted that the wild grey mouse (*Mus musculus musculus*) and an African variety (*Mus musculus gentilis*) developed

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a fatal encephalitis upon intracerebral inoculation, and that another variety (*Mus musculus azoricus*) was susceptible even to intraperitoneal injection of neurotropic virus.

In six years' experience in this laboratory, 100 per cent. of fatal infections has resulted in white mice (*Mus musculus albus*), regardless of their source, upon intracerebral inoculation not only with a neurotropic strain, but even in the first passages in mice using the serum of monkeys infected with the classical French strain. In the Pasteur Institutes of Paris and Tunis, the white mice available in those laboratories proved to be uniformly susceptible to the intracerebral inoculation of a neurotropic strain. Insusceptibility has been found in white mice only in those individuals that have been actively immunized.

Upon various occasions mice have been injected intracerebrally with material of doubtful infectivity, such as mouse brain in high dilution (1 to 1,000,000) or after prolonged ageing. Some animals died of encephalitis, others remained well and susceptible, but none of the survivors was immunized. Under these conditions, an ' all or none ' behaviour resulted with regularity.

On one occasion 20 tame brown mice (*Mus musculus* chocolate variety) were injected intracerebrally with the French strain after 210 passages in mice, and all died in the same manner as the control white mice. However, of 4 adult wild house-mice injected at the same time, 3 died typically and 1 survived. In the following experiments adult white mice have been employed, without resorting to specially bred strains and with considerable assurance of their susceptibility.

TESTS FOR VIRUS BY INOCULATION OF TISSUES

The intracerebral injection of tissues taken from mice after extraneural inoculation of neurotropic virus has thus far failed to produce encephalitis. Two mice were injected subcutaneously and two intraperitoneally with 0·1 c.cm. of a heavy suspension (40 per cent.) of infective mouse brain. One day later, blood from each of these mice was injected intracerebrally into normal mice, and all remained well. In a repetition of this test 6 mice were sacrificed 4 days after inoculation, 3 having been injected subcutaneously and the other 3 intraperitoneally, the specimens taken from each group being pooled for injection. Seventy mice in groups of 7 each were injected intracerebrally with blood and with heavy suspensions of liver, spleen, adrenals and kidney. No encephalitis resulted in any of these animals. Further experiments of this nature have been postponed, but their continuance is indicated in view of the successful results obtained by Sawyer and Lloyd (1931).

Immunization. The development of active immunity in mice which remain apparently well after the extraneural injection of yellow fever virus affords strong evidence of an inapparent infection. The possibility seems remote that the virus, if killed in the animal without multiplication, could produce immunity. Moreover, the available data indicate that if the virus is definitely killed before injection then no immunity develops. The animals which survived the injection of virus remained well and active, and in all respects they appeared perfectly well. It is not known whether they underwent any febrile reaction, the absence of fever being an important feature in the conception of inapparent infection.

Technique. For the neurotropic strain, moderately heavy suspensions of infective mouse brain have been injected subcutaneously or intraperitoneally. Occasionally some animals die in the latter part of the second week or in the third week after inoculation. The diagnosis of these is often impossible. Mice are somewhat cannabalistic, and frequently the brain only of a dead or moribund animal is eaten by the survivors. After an interval, usually of about 3 weeks, the immunity of the surviving animals has been tested by intra-

cerebral injection, using a dilute suspension of virus in order that the test should not be too severe. For this purpose, the brain of a definitely sick or dying mouse is ground in a mortar and diluted with 4 c.cm. of physiological saline containing 10 per cent. of normal serum (rabbit or horse). This suspension is centrifuged till the mass of brain tissue is thrown down. The supernatant fluid is then diluted 100-fold, giving approximately a 1-1,000 dilution. Using a tuberculin syringe graduated to $\frac{1}{32}$ c.cm., about one-half of such a division ($\frac{1}{65}$ c.cm.) has been used for all intracerebral injections in adult mice. Without exception, a control group of 6 or 7 mice has always been injected. Death of all mice in these control groups has occurred unfailingly. Usually several of the controls die as late as the 6th or 7th day after injection, instead of on the 5th day, and this delay indicates that the virus has been moderately well diluted.

During the past three years, experimental studies have occasionally required the use of mice immune to yellow fever. In 1932, a preliminary test suggested that immunization might be effected rather readily. The extraneural injection of neurotropic virus rendered 6 of 7 mice refractory to an intracerebral test. Subsequently the results became irregular. It would be of value to know whether, with the continued passage of the virus from brain to brain of mice, it might in time become more obligately neurotropic and therefore less effective in producing immunity upon extraneural injection. The results that have been obtained in the immunization of mice are compiled in Table I.

TABLE I

Immunization of mice against yellow fever by extraneural injection of neurotropic virus

Experiment No.	No. of mice	Infective mouse brain used for injection				No. of mice which survived after 3 weeks	Intracerebral immunity test	
		No. of previous passages in mice	Strength of suspension	Amount in c.cms.	Route of injection		No. of mice which died in 5 to 10 days	No. of mice which survived after 3 weeks
1	7	125	10 per cent.	0·1	subcut.	7	1	6
2	12	128	10 per cent.	0·2	subcut.	12	6	6
	12			0·2	intraperiton.	12	6	6
3	25	133	25 per cent.	0·1	subcut.	*10	10	0
	25			0·1	intraperiton.	*5	0	5
4	21	152	10 per cent.	0·1	intraperiton.	14	13	1
5	16	180	25 per cent	0·1	subcut.	15	11	4
	16			0·1	intraperiton.	13	10	3

* An interval of 5 weeks was used before the immunity test, on account of the large number of deaths from intercurrent infection.

The controls for these experiments are not shown in the Table. Groups of 6 or 7 mice were used in each experiment, and all these animals died within 5 to 7 days after injection. In the second experiment, the 6 controls were all dead at the end of the 6th day ; of the 12 mice which were not protected by the preliminary injection there were 5 animals in which death was deferred till the 9th or 10th day. A delay of this length is suggestive of a partial degree of immunization. In the third experiment, many animals died of intercurrent infection. However, of the 25 mice injected intraperitoneally, 4 died on the 10th day with symptoms of encephalitis ; in one of them the diagnosis was confirmed by intracerebral injection of brain into normal mice. Encephalitis developed in a similar way in the last experiment. Of the 16 mice injected intraperitoneally, 3 developed paralysis 5 days after inoculation, and one each of these paralyzed mice died on each of the 3 succeeding days.

Immunity in some instances develops promptly. Ten mice were injected subcutaneously with 0·2 c.cm. of a 10 per cent. suspension of infective mouse brain of the 127th passage. The immunity test was given one week later ; seven died within 5 to 6 days and 2 remained well. One animal behaved in an exceptional manner, developing a complete flaccid paralysis of both hind legs and of the tail. Eventually a well-marked but not complete return of function occurred in one leg, but the tail and the other leg remained paralyzed. This animal died 3 months and 2 days after the first injection of virus. During the earlier passages of the classical virus by intracerebral injection, a normal mouse may, as a rare exception, linger for a week or 10 days with paralysis of the extremities, without showing any improvement.

Duration of Immunity. In working with immune mice, it is important to note that the survivors of the intracerebral inoculation do not remain refractory indefinitely. Four mice that survived an immunity test were re-injected intracerebrally $8\frac{1}{4}$ months later, with a 10 per cent. suspension of infective brain without centrifugalization. One of these remained well and 3 died on the 6th, 10th and 12th days respectively after the injection. Even under less severe conditions, the resistance to intracerebral injection was found to be relatively transient. Thus, at a shorter interval after immunization, 14 mice were re-tested intracerebrally with a 1–1,000 dilution of infective brain, using only the supernatant fluid, and only 5 of these animals survived. The results are given in Table II.

TABLE II

Duration of immunity to intracerebral inoculation of virus

No. of mice	Time in days since first immunity test	No. of mice which died after second immunity test. Time in days after injection									No. of mice which lived after 3 weeks
		4	5	6	7	8	9	10	11	14	
8	106	–	–	2	–	1	–	–	1	1	3
6	139	–	–	1	1	1	–	–	1	–	2
7	controls	1	2	3	1	–	–	–	–	–	0

Passive Immunity. The first protection tests in yellow fever were conducted successfully by the members of the French Commission, Marchoux, Salimbeni and Simond (1903). These investigators showed in a conclusive manner that the injection of convalescent yellow fever serum into susceptible volunteers afforded protection against yellow fever. In the comparison of yellow fever in West Africa and South America, Theiler and the writer (1928) found that small amounts of human immune serum protected monkeys against experimental yellow fever, a result which was soon described independently by Hudson, Bauer and Philip (1929). Furthermore, Theiler (1931) applied the protection test to mice, using intracerebral inoculation of neurotropic virus, but his original technique gave results that were subject to some irregularity. Frequently, even a small amount of infective brain (1 to 100 dilution of the supernatant fluid) may mask the protective effect of sera known to be immune. If one uses higher dilutions (such as 1 to 1,000) then the control animals may survive, if physiological saline is used as a diluent.

However, with two minor modifications, Laigret and myself (1932) used the intracerebral technique with very satisfactory results. On one occasion, in doing an intracerebral protection test with several specimens of serum, a control group of mice was injected, at the end of the experiment, with the supernatant fluid from a suspension of brain (1-100) that had stood at room temperature for only two hours. This suspension was made in physiological saline and, in this short time, lost its infectivity completely. After a similar time at room temperature some of this suspension that happened to contain normal serum killed all the 6 mice that were injected with it. The addition of 10 per cent. of normal serum (horse or rabbit) to physiological saline affords a satisfactory diluent for preparing the suspension of infective mouse brain. A similar observation has been recorded by Bauer and Mahaffy (1930) in the preparation of high dilutions of infective monkey serum.

The second precaution is that the dosage of virus must be rather small, e.g., a 1-1,000 dilution of the supernatant fluid as described in the technique for preparing infective brain in testing mice for active immunity by intracerebral injection. Equal parts of the 1-1,000 dilution of virus and of the serum to be tested are mixed, and without incubation a small amount ($\frac{1}{60}$ c.cm.) of the mixture is injected intracerebrally in mice. All the controls die usually within 6 to 7 days. A technique essentially similar to this has been described independently by Theiler (1933). A further improvement has recently been added by Laigret (1934), consisting in a preliminary titration and preservation of the infective brain before carrying out a series of tests.

Protection tests were made with the serum of mice known to be immune and with the serum of others in which the immunity was either partial or doubtful. Three specimens were selected, the first being a pooled sample of the blood of 3 mice which had recently withstood an intracerebral injection of virus. The second was from a mouse which had been injected intraperitoneally with virus ; but after the intracerebral immunity test this animal developed paralysis on the 9th day and was bled at this time. The third specimen was somewhat similar : a mouse, following an intraperitoneal injection of virus, withstood an intracerebral immunity test, but $2\frac{1}{2}$ months later a second intracerebral immunity test resulted in paralysis 8 days later, and the animal was bled at once. Of the three specimens, only the first and third gave evidence of protection. The results are shown in Table III.

TABLE III

Protection tests with the serum of mice for the detection of passive immunization

Condition of mice providing serum	No. of mice injected	No. of mice which died. Time in days after injection						No. of mice which survived after 3 weeks
		4	5	6	7	8	9	
Immune	6	–	–	–	1	–	1	4
Paralyzed after first immunity test ...	6	1	–	–	3	1	1	0
Paralyzed after second immunity test ...	6	–	–	–	–	–	2	4
Normal (control) ...	6	–	–	4	2	–	–	0

EXTRANEURAL INJECTION OF MICE WITH THE CLASSICAL VIRUS

In a preliminary test, 8 adult mice were injected intraperitoneally with the serum (0·2 c.cm.) of a monkey dying of yellow fever. Ten days later, one of these developed complete paralysis of the hind legs and tail and died of encephalitis. The seven surviving mice showed no symptoms. After an interval of 3 weeks, they were injected intracerebrally with neurotropic virus, and all died within 5 to 7 days. The behaviour of the classical virus was then tested with a larger group of normal mice and also with a few immunes.

Infective monkey serum (0·2 c.cm.) was injected into each of 25 mice subcutaneously, and in the same dosage intraperitoneally into another group of 25. Also, 8 mice were injected intracerebrally with this serum to determine whether it was infective in small quantities, and these 8 died within 7 to 10 days.

The extraneural injection of the classical virus resulted in the death of a few animals from encephalitis, and of the survivors only a few were found to be immune. Of the mice injected intraperitoneally 2 died of encephalitis after 9 days, the diagnosis in one being confirmed histologically and in the other by the inoculation of brain tissue into normal mice. Of the subcutaneous group, 1 was dying of encephalitis after 9 days. An inoculation of brain from this animal was made intracerebrally into 5 mice ; 9 days later all of these were definitely sick, 3 being found dead on the next day and the remaining two dying on the following day. One other mouse of the subcutaneous group died with indefinite symptoms on the 21st day after injection. Histologically, the brain showed no evidence of encephalitis. After an interval of 3 weeks, an

immunity test was made with neurotropic virus (1–1,000 dilution of the supernatant fluid). Of the 20 remaining mice in the intraperitoneal group 3 survived, and of the 23 in the subcutaneous group 4 remained well.

The immune mice which were available at this time had $1\frac{1}{2}$ months previously withstood without symptoms an intracerebral injection of infective mouse brain (1–1,000 dilution of the supernatant fluid). Thirteen of these immunes were injected intracerebrally, 8 being given infective monkey serum and 5 receiving a 10 per cent. suspension of infective brain without centrifugalization. Of the 8 immunes receiving the classical virus, 2 became paralyzed and died, one on the 5th and the other on the 8th day after injection ; the other 6 remained well. Of the 5 immunes injected with a heavy suspension of neurotropic virus, 4 died within 4 to 6 days and the remaining one on the 16th day after injection. These data are summarized in Table IV.

TRAUMATIZATION OF THE BRAIN FOR THE DETECTION OF INAPPARENT INFECTION

After traumatizing the brain of mice and then injecting intraperitoneally the neurotropic virus of yellow fever, Sawyer and Lloyd (1931) were successful in producing encephalitis. For the purpose of producing trauma, the usual technique of injecting starch paste resulted in a high proportion (83 per cent.) of infections. This technique of injuring the brain has proved of interest in testing mice for inapparent infection with yellow fever ; several days after the extraneural injection of virus, starch paste was injected intracerebrally, and a considerable number of the animals developed encephalitis.

Technique. A 2 per cent. solution of commercial corn starch was prepared by the usual routine of making a thin paste of starch in cold water and then pouring this slowly into boiling water. This was sterilized in the autoclave. Water rather than physiological saline was used since injury of the brain is desired.

A moderately large group of mice (83) were injected with neurotropic virus. A 15 per cent. suspension of infective mouse brain was prepared in physiological saline containing 10 per cent. of normal horse serum, and this was injected in 0·2 c.cm. amounts into each of the mice, one half of the group being injected intraperitoneally and the other half subcutaneously. These two groups were subdivided into smaller lots, usually of 6 mice each. On each successive day for 6 days, one lot from each group was injected intracerebrally with a small amount ($\frac{1}{60}$ c.cm.) of starch paste. Two control lots received an intracerebral injection of starch just before the intraperitoneal and the subcutaneous injection of virus. The animals which ultimately survived were given an intracerebral immunity test 23 days after the extraneural injection of virus, using a 1-1,000 dilution of the supernatant fluid from infective mouse brains.

The first test was made with virus from the 189th passage. Of the group inoculated intraperitoneally, only a few animals died among those which received starch on the 1st and 2nd day after the injection of virus. A large number of the intraperitoneal group (15 of 41) died during the first two weeks after the injection of starch, but only a small number of survivors (6) proved to be immune. Of those inoculated subcutaneously, a small number (6 of 41) died within 2 weeks after the injection of starch but a large number (18) were immunized. The details of these results are shown in Tables V and VI.

TABLE IV

Effect of the intraperitoneal and subcutaneous injection of mice with the classical virus of yellow fever

Mice used for injection		Classical virus				Neurotropic virus	
No.	Condition	Mode of injection of infective monkey serum	No. which died of encephalitis	No. which died of unknown cause	No. which remained well	Effect of intracerebral injection of infective mouse brain*	No. of mice which were well after 3 weeks
25	normal	intraperitoneal	2	3	20	17 died of encephalitis	3
25	„	subcutaneous	1	1	23	19 „ „	4
8	„	intracerebral	8	0	0	—	–
8	immune	intracerebral	2	0	6	—	6
5	immune	—	–	–	–	all died of encephalitis	0
5	normal	—	–	–	–	„ „	0

* A 1,000 dilution was used, except for the group of 5 immunes which were injected with a 10 per cent. suspension

TABLE V

Effect of the intracerebral injection of starch in mice on successive days after the intraperitoneal injection of neurotropic virus

No. of mice injected	Injection of starch. Time in days after injection of virus	No. of mice which died. Time in days after injection of starch										No. of mice which survived	Intracerebral immunity test of survivors. No. of mice which died. Time in days								No. of mice which survived after 3 weeks
		1	2	3	4	5	6	7	8	13	22		6	7	8	9	10	11	12	13	
5	0	–	–	–	–	–	1	1	–	–	–	3	1	–	1	–	–	–	–	–	1
6	1	–	–	–	–	2	–	–	–	–	1	3	–	–	1	–	1	–	–	–	1
6	2	–	–	–	–	–	–	–	1	–	–	5	–	1	1	2	–	–	–	–	1
6	3	–	–	–	1	1	1	–	2	–	–	1	–	–	–	–	–	–	–	–	1
7	4	–	1	1	–	–	–	–	–	1	–	4	–	1	–	1	–	–	–	1	1
6	5	–	–	1	–	–	1	–	–	–	–	4	–	2	–	1	–	–	–	–	1
*5	6	–	–	–	–	–	–	–	–	–	–	5	–	–	1	1	2	–	1	–	0
		Controls										7	4	3	–	–	–	–	–	–	0

* One mouse intended for this group was dying of encephalitis on the 6th day after injection of virus

TABLE VI

Effect of the intracerebral injection of starch in mice on successive days after the subcutaneous injection of neurotropic virus

No. of mice injected	Injection of starch. Time in days after injection of virus	No. of mice which died. Time in days after injection of starch								No. of mice which survived	Intracerebral immunity test of survivors. No. of mice which died. Time in days									No. of mice which survived after 3 weeks
		5	6	7	12	18	19	23	24		5	6	7	8	9	10	11	12	13	
6	0	–	1	2	–	–	–	–	1	2	–	1	–	–	–	1	–	–	–	0
5	1	–	–	–	–	–	–	1	–	4	–	–	–	1	–	–	–	–	–	3
6	2	–	–	–	–	–	–	–	–	6	–	–	1	2	–	–	1	–	–	2
6	3	–	–	–	–	–	–	–	–	6	1	–	1	–	–	–	–	–	1	3
6	4	–	1	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	5
6	5	–	–	–	1	–	1	–	–	4	–	–	2	–	–	–	–	–	–	2
6	6	1	–	–	–	1	–	–	–	4	–	–	–	–	–	1	–	–	–	3
		Controls								7	–	4	3	–	–	–	–	–	–	0

This experiment, using infective mouse brain of the 200th passage, was repeated with somewhat similar results, the immunity test of the survivors being given 31 days after the extraneural injection of virus. As before, in the intraperitoneal group a large number of deaths (22 of 44) occurred during the first 2 weeks after the injection of starch, though only a few (3 of 13) died of those which received starch on the 1st or 2nd day after the injection of virus. This is suggestive, though not definite evidence, of the duration of the period of incubation.

In this second test, several of the intraperitoneal group died within 5 to 7 days after the extraneural injection of virus, but only 1 to 3 days after the injection of starch. However, the majority of these animals developed definite symptoms of encephalitis, the virus having already localized in the brain before the intracerebral injection of starch.

As in the previous test, the subcutaneous group showed only a few deaths (3 of 44) during the first two weeks following the starch injections. Subsequently 7 others died of undetermined causes, and of the 34 which remained there were 15 which proved to be immune. The data are given in more detail in Tables VII and VIII.

The neurotropic strain of yellow fever produced by Theiler (1930) in mice was found by the writer (1931) to cause a fatal encephalitis upon intracerebral injection in normal monkeys (*Macacus rhesus*), but not in monkeys immune to the classical strain of yellow fever. The monkeys dying of encephalitis failed to show the usual necrosis of the liver. Similar observations were made by Lloyd and Penna (1932). Furthermore, the neurotropic strain infects mosquitoes (*Aëdes aegypti*) either with difficulty or not at all. This biological modification of the virus expresses itself in the form of a new disease in the sense of producing a type that has not hitherto been recognized. Nevertheless it would be premature to assume that neurotropic yellow fever is a purely artificial condition which never occurs in nature. However, one could hardly expect to find neurotropic yellow fever developing in rhesus monkeys if exposed to infection under natural conditions, since even the intracerebral injection of the classical virus in normal monkeys produced no encephalitis but resulted in typical necrosis of the liver. One might consider the possibility of the development of encephalitis in rodents, for example, during an epidemic of yellow fever ; if any such infections occur, they would, under ordinary circumstances, escape suspicion of being associated in any way with this disease.

SUMMARY

White mice were injected intraperitoneally and subcutaneously with the neurotropic and with the classical virus of yellow fever. A small proportion of the animals died of encephalitis. Of the survivors, it appeared that in some instances the virus died without producing any effect, the animals remaining susceptible ; in other cases an active immunity developed.

TABLE VII

Effect of the intracerebral injection of starch in mice on successive days after the intraperitoneal injection of neurotropic virus
(Repetition of the experiment in Table V)

No. of mice injected	Injection of starch. Time in days after injection of virus	No. of mice which died. Time in days after injection of starch															No. of mice which survived	Intracerebral immunity test of survivors. No. of mice which died. Time in days											No. of mice which survived after 3 weeks
		1	2	3	4	5	6	7	8	9	10	11	13	17	20	25		1	2	3	4	5	6	7	8	12	14	15	
7	0	–	–	–	–	1	–	1	1	–	1	1	–	–	–	–	2	–	–	–	–	–	–	–	1	–	–	1	0
6	1	–	–	–	–	1	–	–	–	–	1	–	–	–	–	–	4	–	–	–	–	–	–	–	3	–	–	–	1
7	2	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	6	–	–	–	–	–	2	1	3	–	–	–	0
6	3	1	1	1	–	–	–	–	–	–	–	–	–	1	1	–	1	–	–	–	–	–	1	–	–	–	–	–	0
7	4	–	1	1	–	–	–	1	–	–	–	–	1	–	–	–	3	1	1	–	–	–	–	–	–	1	–	–	0
6	5	1	1	–	–	2	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	1	–	–	–	–	–	–	0
5	6	1	–	–	1	–	–	–	1	–	–	–	–	–	–	–	2	–	–	–	–	–	–	1	–	–	1	–	0
		Controls															7	–	–	–	–	1	3	3	–	–	–	–	0

TABLE VIII

Effect of the intracerebral injection of starch in mice on successive days after the subcutaneous injection of neurotropic virus (Repetition of the experiment in Table VI)

No. of mice injected	Injection of starch. Time in days after injection of virus	No. of mice which died. Time in days after injection of starch												No. of mice which survived	Intracerebral immunity test of survivors. No. of mice which died. Time in days														No. of mice which survived
		1	2	3	4	5	6	7	8	16	18	24	27		2	3	4	5	6	7	8	10	11	12	14	16	17	20	23	24	
6	0	–	–	–	1	–	1	–	–	–	–	–	–	4	–	1	–	–	–	–	1	–	–	–	–	–	–	–	–	1	1
6	1	–	–	–	–	–	–	–	–	1	–	–	–	5	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	3
7	2	–	–	–	–	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	1	–	–	–	–	1	1	–	4
7	3	–	–	–	–	–	–	–	1	–	–	–	2	4	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–	–	2
7	4	–	–	–	–	–	–	–	–	1	–	1	–	5	–	–	–	–	–	–	1	1	–	1	1	–	–	–	–	–	1
5	5	–	–	–	–	–	–	–	–	–	1	–	–	4	1	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	2
6	6	–	–	–	–	–	–	–	–	–	–	1	–	5	–	–	–	–	–	–	–	2	–	–	–	–	1	–	–	–	2
		Controls												7	–	–	–	1	3	3	–	–	–	–	–	–	–	–	–	–	0

In order to test for the presence of virus in the blood-stream, sterile starch paste was injected intracerebrally in mice at intervals of several days after injection of neurotropic yellow fever virus, the intraperitoneal route being used in one group of animals and the subcutaneous route in another. Of the intraperitoneal group, a considerable proportion died of encephalitis and only a small number of the survivors were immune to an intracerebral injection of virus. Of the subcutaneous group, a small number of animals died of encephalitis but many of the survivors were immune. The interpretation of these results seems to be reasonably clear. After intraperitoneal inoculation there was frequently an inapparent infection with invasion of the blood-stream; also subcutaneous inoculation often led to inapparent infection, but with less tendency to invasion of the blood. Indeed, it is significant that traumatization of the brain, after almost massive subcutaneous injections of virus, was attended by relatively little danger of the development of encephalitis.

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ANOTHER ANOPHELINE OF THE *FUNESTUS* SERIES FROM SOUTHERN RHODESIA

BY

H. S. LEESON

(*London School of Hygiene and Tropical Medicine*)

(*Received for publication* 31 *January*, 1935)

The anopheline described here was observed during a visit to Southern Rhodesia, and was separated in the larval stage because of a superficial resemblance to the larva of *A. longipalpis*. When adults were bred out they appeared to be much more closely related to *A. funestus*. Larval and pupal pelts, together with their respective adults, have recently been examined, as also have some preserved larvae and pupae.

Other larvae, about which there was no question of their being *A. longipalpis*, gave rise to typical adults of that species, though some of them had a pale area on the third vein and others the usual completely dark third vein (De Meillon, 1931).

The different stages of the two anophelines were then compared, and later those of both compared with those of the *funestus* series described by Evans and Leeson (1935). So far as the resemblance to *A. longipalpis* is concerned, it was found that the chief likeness in the larvae was in the shape and size of the main tergal plates ; but the accessory tergal plates, which are single on segment II and are three in number on segments III—VII in *A. longipalpis*, are single in the new larva on segments II and III and triple on V—VII ; on one pelt there are three accessory plates on segment IV also, but on another only one of the paired plates is to be seen. In the larva itself, the paired posterior accessory tergal plates are often difficult to see. Other characters, however, serve to distinguish it very readily from that of *A. longipalpis*. On the head, for instance, the fronto-clypeal markings include an anterior transverse band such as is found in *A. funestus*, while in this position in the larva of *A. longipalpis* there are two submedianly placed dark spots, which may occasionally be joined to form a broad band. The post-frontal hairs, moreover, are like those of *A. leesoni*, that is, branched from 3 to 8 times. In *A. longipalpis* this hair is simple (occasionally bifid), as in *A. funestus*. Another distinction between this larva and that of *A. longipalpis* is found in the lateral saddle hair on the anal segment, which in the former is simple, as in *funestus* series, while that of the latter is either bifid or trifid (De Meillon, 1933).

With regard to the pupa, the accessory paddle hair of the new anopheline is branched as in *leesoni*, but the same hair in *A. longipalpis* is always simple as in *A. funestus*. Spine A in both species resembles that in *leesoni* ; hairs B and 4 are similar to those of *funestus*. Hair C is simple in both (sometimes bifid

in the new species), though longer in the new anopheline than in *A. longipalpis*, in which it resembles *A. leesoni*.

As has already been noted, the adults are in no way like those of *A. longipalpis* but are nearer to those of the *funestus* series. The base of the club of the male palp is dark, as in *funestus*. The terminalia of the male are of the *A. leesoni* type, while in the female the hair-like processes of the post-armature ridges of the pharynx are definitely longer than those of *A. leesoni* and approach those of *A. funestus* var. *confusus* in length. The third vein is all dark (Leeson, 1930).

It is thought advisable for the present to regard this anopheline as a variety of *A. funestus* until such time as the status of the various members of the series has been thoroughly worked out. A detailed description follows.

Anopheles funestus var. **rivulorum** var.nov.

ADULT. A small dark anopheline having *funestus*-like characters.

♀. *Head.* Length of proboscis 1·4–1·7 mm. (6 specimens). *Palps.* Three pale bands, apical pale band a little wider than other two.

Pharynx. Hair-like processes on post-armature ridges short, as in *A. funestus* var. *confusus.*

Thorax. Mesonotum with hairs ; scales present only in usual anterior situation.

Legs. All dark.

Wings. Length 2·8–3·0 mm. (6 specimens). Basal third of costa all dark, no pale interruption ; 3rd vein all dark ; pale scales at bifurcations and cross-veins ; large dark fringe spot opposite 2·2 ; pale fringe spots opposite the other veins except 6 ; remainder of wing as in *A. leesoni.*

♂. *Head.* Length of proboscis 1·7–1·9 mm. (4 specimens). *Palp.* Club with two pale areas as in *A. funestus* ; base of club dark.

Wing. Length 2·6–3·1 mm. (4 specimens).

Terminalia. As in *A. leesoni.*

PUPA. *Paddle.* Accessory hair branched 3 or more.

Spine A. IV, about 1/3 length of segment ; V—VII, about half or little more than half length of segment.

Hair B. IV—VII, 4 to 7 branches.

Hair C. IV, 5–7 branches ; V—VII, simple, longer than one segment.

Hair 4. IV—VII, 3 or 4 branches.

LARVA. *Head.* A dark transverse band, as in *A. funestus*, on the fronto-clypeus. *Post-frontal hairs.* Three or more branches, as in *A. leesoni.*

Thorax. Palmate hairs. Rudimentary. *Mesopleural hairs.* Both anterior dorsal and anterior ventral simple.

Abdomen. Palmate hairs. Segment I, not fully developed, smaller than on subsequent segments, leaflets usually with filaments ; segments II—VII, fully developed, leaflets with abrupt shoulders and filaments. *Tergal plates.* Main, wide from side to side, narrow from back to front, resembling *A. longipalpis.*

Accessory tergal plates : I, absent ; II and III or II—IV, one ; IV—VII or V—VII, three, as in *A. longipalpis*. *Ventral spicules*. Absent. *Saddle hair*. Simple as in *A. funestus* series.

EGG. Not known.

HABITAT. Adults along streams, in crevices and cavities in the banks. Larvae in slowly moving streams near banks and among boulders, collected with *A. funestus* var. *confusus*, *leesoni*, *longipalpis* and *pretoriensis*.

DISTRIBUTION. Type ♂ with larval and pupal pelts and type ♀ from Muzari river, Sinoia (3,870 ft.), January 5th, 1934; other material from the same source and from Nyaderi (3,000–4,000 ft.), March, 1934, all taken and bred by the writer. Types in British Museum. Other material in the collection of the London School of Hygiene and Tropical Medicine.

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THE BRITISH SPECIES OF THE SUBFAMILY SARCOPHAGINAE, WITH ILLUSTRATIONS OF THE MALE AND FEMALE TERMINALIA

BY

W. S. PATTON

AND

COLBRAN J. WAINWRIGHT, F.R.E.S.

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 11 *February*, 1935)

INTRODUCTION

The subfamily Sarcophaginae contains many species distributed throughout all the zoological regions of the world. Most of them are relatively large flies of a characteristic grey colour and facies. Owing to the habit of many of the species of breeding in carrion, they are commonly known as ' flesh flies.' The majority are viviparous, usually depositing their first-stage larvae in large numbers. Many species are common domestic insects, and some have the habit of depositing their larvae in human and animal tissues ; as their larvae are very large and burrow deeply, they cause much destruction of tissue.

As is now known, the ♂♂ can readily be identified by drawing back the anal cerci and exposing the terminalia, the characters of which are, as a rule, strikingly different in each species ; but it is very difficult to identify the ♀♀. One of the writers recently described the segmentation of the abdomen of the ♂ *carnaria* L., as well as the terminalia of this species and of its close ally *vicina* Vill. It was also shown that the ♀ *carnaria* can readily be distinguished from the ♀ *vicina* by noting that tergum 7 is present in the former but wanting in the latter. The other author has recorded the British species in a recent publication, in which a key was given for their identification. It was pointed out there that, in order to facilitate the identification of the ♂♂, a series of drawings of the terminalia was necessary. We propose, therefore, to illustrate the ♂ terminalia of the British species, accompanying the illustrations with the briefest possible descriptions of the parts, for we feel that an accurate drawing is worth pages of description. The terminalia of any of the ♀♀ known for certain (either bred or taken *in cop.*) will also be illustrated. We shall also give the salient diagnostic characters of the adults based on external characters of known value, together with some notes on their distribution, habits, etc. When all the species have been described—and no particular order will be followed—we shall give keys for their identification. We should like to take this opportunity of enlisting the help of collectors in this country to let us have any pairs taken *in cop.*, or ♂♂ and ♀♀ bred from known larvae.

A few words of explanation regarding the drawings, etc., are necessary. The drawings of the terminalia are not done to scale, but rather for convenience for reproduction ; this in no way detracts from their value. No attempt will be made to describe the complicated phallosome, as this would serve no useful purpose. We must confess that we do not understand why, in certain species, this organ has many bilaterally symmetrical gadgets (we can suggest no better word). The genital opening in the ♀♀ so far examined is very large, even in species in which the phallosome is relatively simple. We have noted, however, that in some species with many gadgets the ♀ has a chitinous plate (signum) on the uterus ; whether there is any connection between the two we cannot say at present. The exact size and shape of the sclerites (especially the sterna) of the larvipositor cannot be made out accurately in whole mounts, as illustrated in the several drawings (fig. 3, *a*, etc.), for in this position the plates appear foreshortened, etc. The sterna and terga have, therefore, been dissected off, mounted flat and drawn at the sides ; this will explain the discrepancies in their appearance in the two drawings (e.g., the sixth and seventh sterna in fig. 3, *a* and *b*).

The abbreviation 'd.c.' stands for dorsocentral, 'ac.' for acrostichal bristles. References to literature will be given at the end of the last part of this series.

Sarcophaga falculata Pandellé. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. *S. falculata* is one of the larger species of the genus (all the species of *Sarcophaga* vary considerably in size), comparing in that respect with *carnaria* L., *vicina* Vill. and *scoparia* Pand., from all three of which it is immediately distinguished by its red tenth tergum ; it thus comes in the group of species with the terminal segments more or less red. Having bare first longitudinal vein and four or five d.c., of which, however, only the two hinder are strongly developed, it is thus restricted to a small group, the other British species of which are *haemorrhoidalis* Mg. and *tuberosa* Pand. var. *exuberans* Pand. It has a long and strong pair of ac. in front of the scutellum, which is quite lacking in *haemorrhoidalis*, from which it is thus easily separated ; but although these bristles are not so long and strong in *tuberosa* it is difficult to separate it from that species without examination of the terminalia, which are very distinct. *Falculata* has a few short bristles on the hind margin of the seventh tergum, *tuberosa* only fine hairs ; *falculata* has very hairy genae, with many short stiff hairs, but none strong enough to be called bristles ; *tuberosa* has very few fine hairs, but a short row of bristles ; further, *tuberosa* seems to have less pilosity on the legs, especially on the hind pair. *Falculata* has front femora densely short-haired, with the usual ventral and dorsal bristle rows standing out conspicuously ; mid femora with long dense ventral pilosity on basal two-thirds, on the post-ventral apical third a strong comb-like row of thick short thorns (Enderlein's ctenidium), but no other post-ventral bristles, the antero-ventral series represented by an irregular, almost complete, row of short thick

bristles scarcely forming a comb at the apex. The hind femora also densely pilose inside with few bristles, the pilosity being as long as femora are wide, or nearly so ; no post-ventral bristles, and the antero-ventral row irregular, small and scattered from before middle to apex ; the hind tibiae with long and dense internal pilosity. *Tuberosa* seems to have rather more bristles in each series but less pilosity, that on hind femora in particular being little more than half width of femora in length. It should be noted, however, that all these leg characters are very variable. *Falculata* has silvery tomentum on the face and much silvery-white pubescence on the buccae and occiput ; *tuberosa* tends to golden tomentum and less pubescence.

The species of this group are characterized by greatly reduced chaetotaxy on the thorax, only the supra-alar series and the setae immediately in front of the scutellum being well developed, the remainder being small or missing. There are no hind marginal setae on the second segment. *Falculata* has a pair of crossed apical scutellars ; sternites with only very short decumbent hairs. Third section of costa is longer than fourth and fifth together.

♀. The ♀♀ are to be distinguished by the same characters as the ♂♂, but are not easily separated from those of *tuberosa* ; perhaps, however, *falculata* can be recognized by its silvery face and by the abundance of white hair about the buccae and occiput with but slight admixture of black hairs, by numerous short genal bristly hairs, and by the long, strong pair of prescutellar ac.

Notes. *Falculata* was not known as British until Professor J. W. Carr, of Nottingham, sent one of the writers (C.J.W.) a specimen for identification in 1917. This was apparently taken in Nottingham by Mr. F. M. Robinson. In 1927, a Rhodes scholar, McCulloch, took four in a fly-trap in the University Park, Oxford, since when a number have been taken or bred at Oxford by Mr. J. Collins and Mr. A. H. Hamm, mostly, we believe, from fly-traps in the Park or in the museum grounds. In 1928, Professor P. A. Buxton bred a lot of Sarcophagas from meat exposed on the roof of the London School of Hygiene and Tropical Medicine, which proved to be a mixture of *carnaria* and this species ; and more recently Mr. H. Britten has bred it from a dead owl on the roof of the museum in Manchester. Finally one of us caught one on his study window in Birmingham. The fact that all these occurrences were in or about towns seems to point to the possibility of some association with town life, perhaps with the meat-markets, suggesting that the species has been introduced with meat. The only exception to the above is a single ♂ taken by Mr. J. Collins at Yarnton, near Oxford, on *Heracleum sphondylium* L. Mr. J. E. Collin reports a pair in the Verrall collection bred in 1873 from puparia found in dead pigeons in a pigeon-loft, probably in London, and it is possible that the inclusion of *nurus* Rond. in the Verrall list was due to these. The species was described by Pandellé from Wiesbaden, and he recorded it also from other parts of Germany and from various places in the Mediterranean region. Lundbeck adds North Africa and North India, and Aldrich records it from U.S.A. and from Hawaii,

which distribution again suggests artificial introduction through meat or some similar cause.

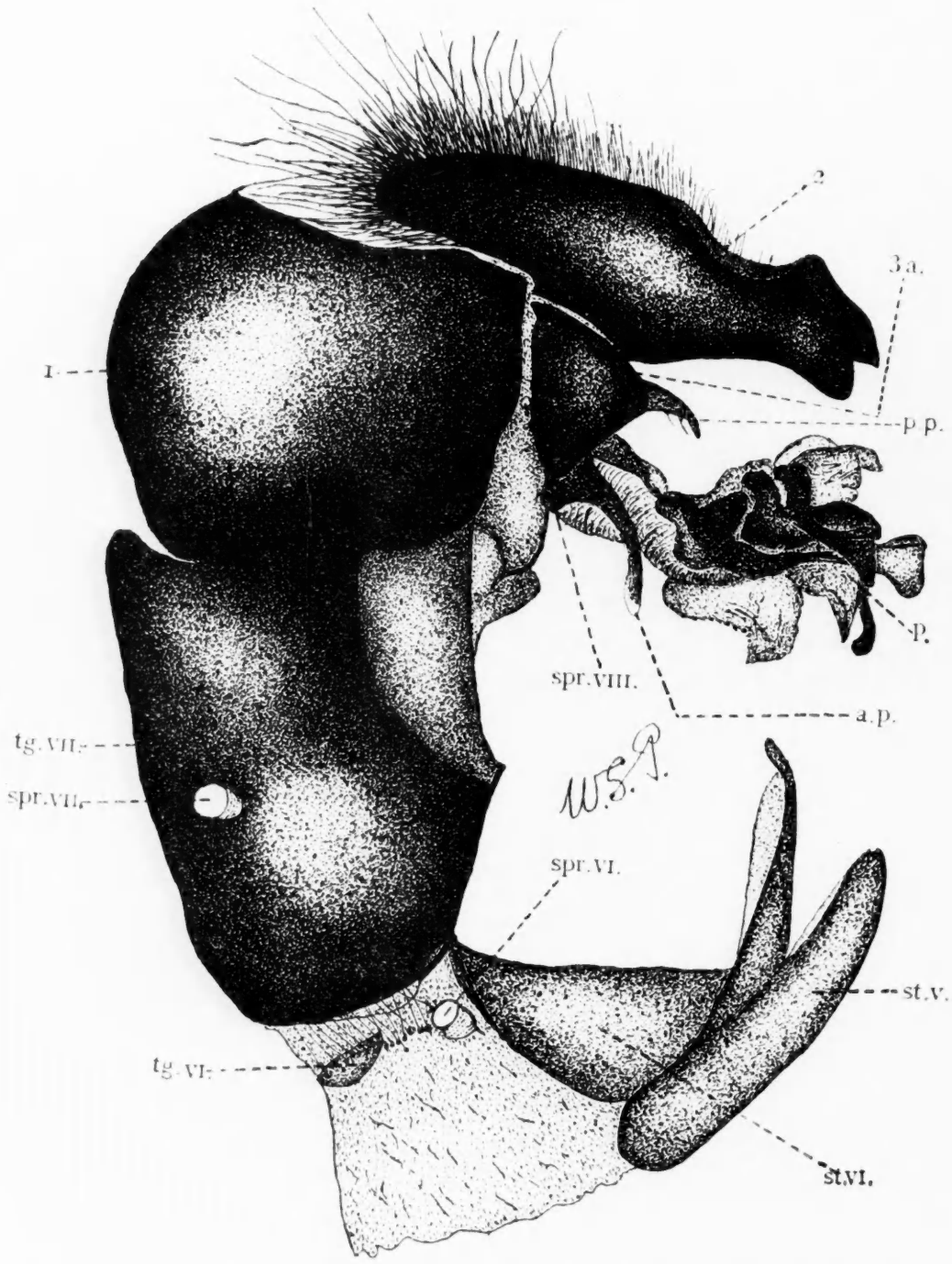

FIG. 1. Terminalia of ♂ *S. falculata* in side view. *st. v, st. vi.*—Fifth, sixth sterna ; *spr. vi, spr. vii, spr. viii.*—Sixth, seventh, eighth spiracle ; *tg.vi, tg. vii.*—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a.*—Distal segment of ninth coxite ; *a.p.*—Anterior paramere ; *p.*—Phallosome ; *p.p.*—Posterior paramére (British specimen).

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. The terminalia, as seen in side view when the anal cerci are drawn back, are illustrated in fig. 1 ; the parts are fully labelled. GENERAL CHARACTERS. Large, red ; anal cerci covered with numerous hairs posteriorly. SCLERITES. Sternum 5 (fig. 1, *e*)

large, Y-forked, the inner surface of forks armed with numerous stiff hairs on dense chitin. Tergum 6 (fig. 1) two small plates on each side of middle line ; sternum 6 (fig. 1) a large bent plate firmly attached to dorsal surface of sternum 5. Tergum 7 (fig. 1) a long wide plate. Ninth tergo-sternum (fig. 2, *f*) a short wide plate, the posterior arms relatively short, curving in but not meeting. Tergum 10 (fig. 1) a short, wide, rounded plate.

APPENDAGES. *Ninth Coxite.* Ventral view. *Distal segment.* As in fig. 2, *b* ; note the sclerosed 8th spiracle close to it. Lateral view. Figs. 1, 3*a* ;

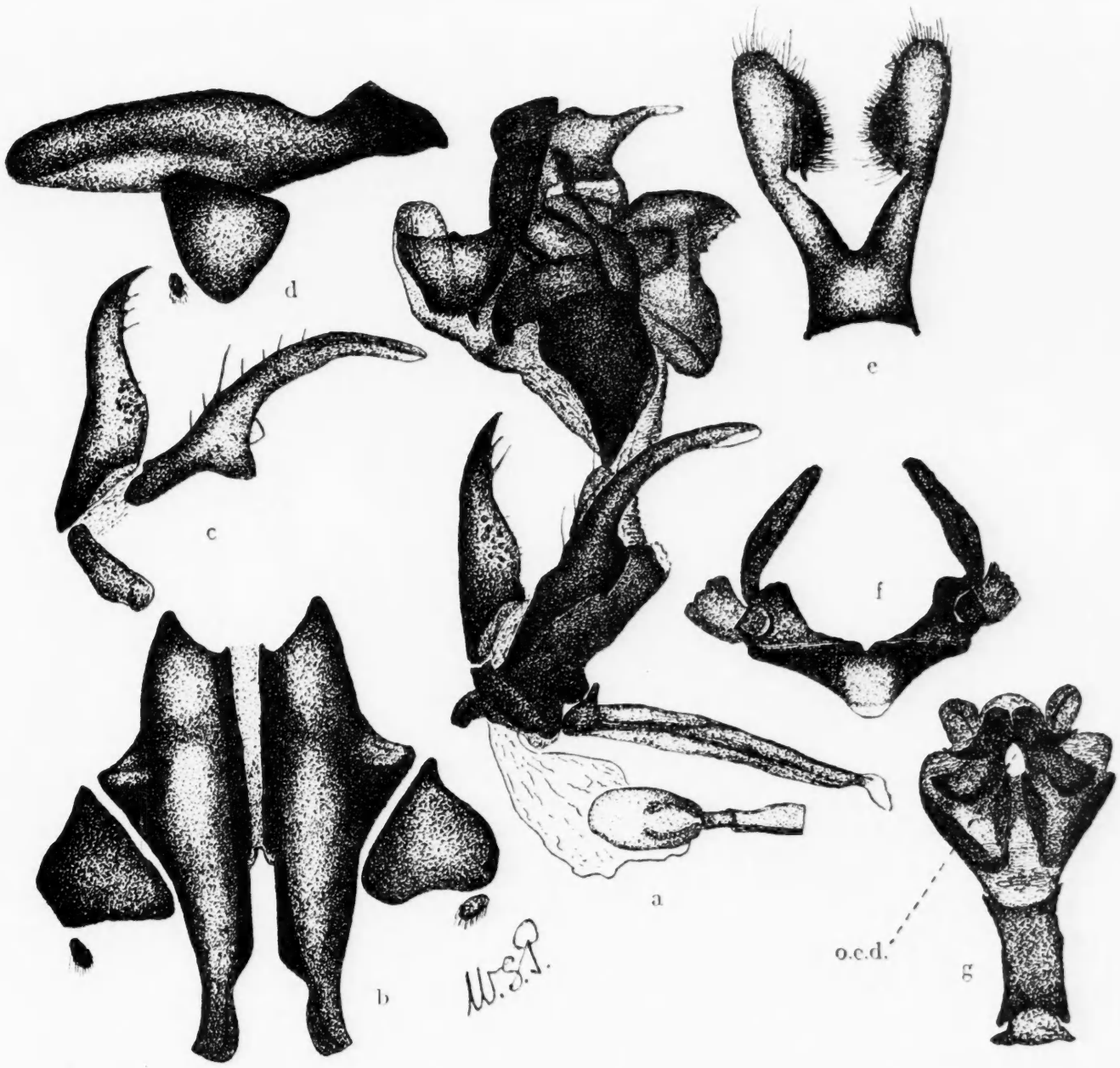

FIG. 2. *a.*—Phallosome and one paramere of *falculata* in side view ; *b.*—Ventral view of anal cerci and distal segments of ninth coxite ; *c.*—Right paramere of same showing two parts ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Fifth sternum ; *f.*—Ninth tergo-sternum ; note its width and very short handle ; *g.*—Dorsal view of phallosome ; *o.e.d.*—opening of ejaculatory duct (British specimen).

2, *d*. A large triangular plate. *Anal cercus*. Ventral view. As in fig. 2, *b*, about anterior half free, remainder joined to fellow. Lateral view. Fig. 2, *d*. A short, wide, rod-like plate indented ventrally near end, then raised, and ending in a blunt point (depending on angle of view).

Phallosome. Lateral view. Figs. 1 ; 2, *a*. The distal part a massive structure with numerous bilaterally symmetrical gadgets ; attached mainly by membrane to long, rather narrow, proximal part. The dorsal view (fig. 2, *g*) shows the opening of the ejaculatory duct. *Posterior Process of Phallosome*. Fig. 2, *a*. Very short and curved. *Sperm Pump Sclerite*. Fig. 2, *a*. Large, as in most species of *Sarcophaga*. *Apodeme of Phallosome*. Fig. 2, *a*. Long. *Parameres*. Lateral view. *Anterior Part*. Fig. 2, *a*, *c*. A long curved rod, slightly expanded at end with a row of short hairs along curved ventral border. *Posterior Part*. Fig. 2, *a*, *c*. A short, sharply pointed, upstanding rod with several short spine-like bristles near end and a patch of sensory hairs.

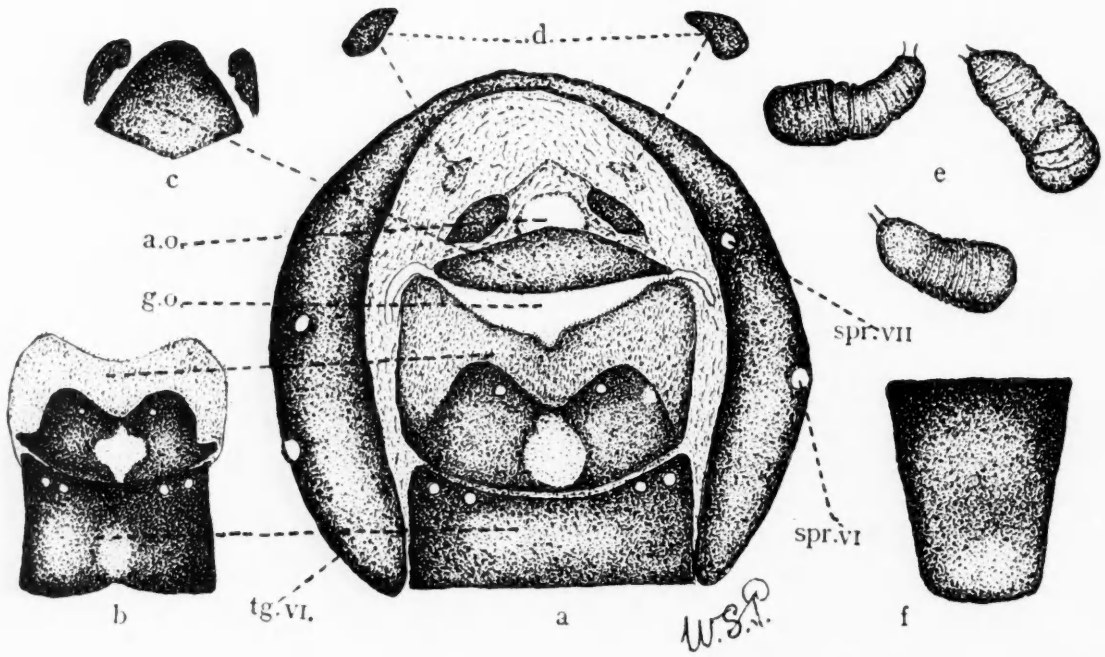

FIG. 3. *a*.—Ventral view of terminalia of ♀ *falculata* to show diagnostic characters ; *spr. vi*, *spr. vii*.—Sixth, seventh spiracle ; *tg. vi*.—Sixth tergum ; *a.o*.—Anal opening ; *g.o*.—Genital opening ; *b*.—Sixth, seventh sterna of same ; *c*.—Tenth sternum and anal cerci of same ; *d*.—Seventh terga of same ; *e*.—Spermathecae of same ; *f*.—Signum of uterus of same (British specimen).

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. LARVIPOSITOR. SEGMENTS 6, 7, 9 and 10. Fig. 3. GENERAL CHARACTERS. Yellow. SCLERITES. Fig. 3, *a*. Tergum 6 consisting of a single plate ; sternum 6 a wide quadrilateral plate, usually with a pair of stout, backwardly directed bristles at each side. Tergum 7 two small widely separated plates in membrane between anal cerci and tergum 6 ; sternum 7 a large plate, the strongly chitinized part (when cleared in caustic potash) resembling two birds' heads with their beaks in contact, the calyx (scar) of the single bristle being the eye of each ; the

enclosed lighter oval area always stands out as a yellowish, shining patch, remainder of sternum lightly chitinized and covered with fine hairs. The ninth tergum and sternum appear to be wanting in this species. Tergum 10 is also wanting. *Anal cerci.* A pair of small curved plates, one on each side of the anal opening. Sternum 10 is a triangular plate. *Spermathecae.* As in fig. 3, *e*. *Chitinous Plate of Uterus* (signum). Fig. 3, *f*. Long, wide and folded anteriorly. The important diagnostic characters of the ♀ terminalia are their yellow colour, tergum 6 consisting of a single plate, the shape and structure of sternum 7, and the characteristic large, central, shining, yellowish spot in the middle of it.

Sarcophaga crassimargo Pandellé. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. *S. crassimargo* Pand. is a medium-sized species usually 8–9 mm. long, though varying greatly in this respect after the habit of the members of the genus. It has black terminal segments ; no setae on the first longitudinal vein ; three strong postsutural d.c. ; no presutural ac.; and marginal setae on the apparent second abdominal segment. It is a species that can be recognized fairly easily from the fact that the usual depression on the first abdominal segment is not continued to the hind margin, leaving a definite flat space, hence its name. However, it closely resembles *agnata* Rond. and *rosellei* Böttch., and for certain identification the terminalia should be examined. *Agnata* has a narrower frons, never more than equal to two-fifths the width of an eye ; in *crassimargo* and *rosellei* the frons is about three-fifths of eye width. *Crassimargo* has a row of postero-ventral bristles on the mid femora running from base to tip and of similar strength throughout, becoming rather shorter towards the tip but never forming a comb of short strong setae ; *agnata* has this row from the middle to the tip, consisting of short, stout, thorn-like setae, forming the characteristic comb (Enderlein's ctenidium) ; and the hairs on the basal portion are sharply contrasted, being long and fine. *Rosellei* agrees with *crassimargo* in both the above respects, but on the hind femora the latter has the postero- and antero-ventral rows of bristles unusually developed, being complete, dense and very long, the postero-ventral row in particular including many hairs much longer than the femur is wide ; in *rosellei* these two series are much less developed, being barely equal to width of femur and being particularly short in the basal portion. *Filia* Rond. is another similar species, but besides having a much wider frons, approaching eye width at vertex, its hind femora have no conspicuous rows of bristles, the usual series consisting of fine hairs not very long.

Crassimargo has strong apical scutellary bristles, well-developed brushes on the edges of the apical sternite, and long fine hairs on hind tibiae in antero- and postero-ventral series (but these are especially variable throughout the genus, sometimes almost wholly failing in weakly-developed individuals). The antero-ventral bristles on the mid femora consist of a few strong, long ones in basal half, and on apical half a finer, shorter series sharply contrasted.

♀. The ♀♀ of this group are especially difficult to separate. *Crassimargo* can perhaps be recognized by the wide, flat, hind margin to the first segment; but it is not so well shown in the ♀♀ as in the ♂♂, and ♀♀ of other species do not show the contrary so well. Its frons is fully eye width, and that of *agnata* definitely less ; the combs on the antero- and postero-ventral aspects of the mid femora seem well developed in *crassimargo*, and extend from the middle to the tip, whilst in *rosellei* they seem less comb-like and weaker, and appear to extend along barely the apical third. The abdomen of *crassimargo* in this sex is very flattened and rather broad.

Notes. This is one of the common British species. We have it from as far north as Forres, and it probably occurs everywhere. According to Böttcher it would appear to be similarly widely distributed and common on the Continent, being recorded from France, Germany, Italy and Greece, and Lundbeck adds Sweden (Ringdahl) and Finland, as well as Denmark.

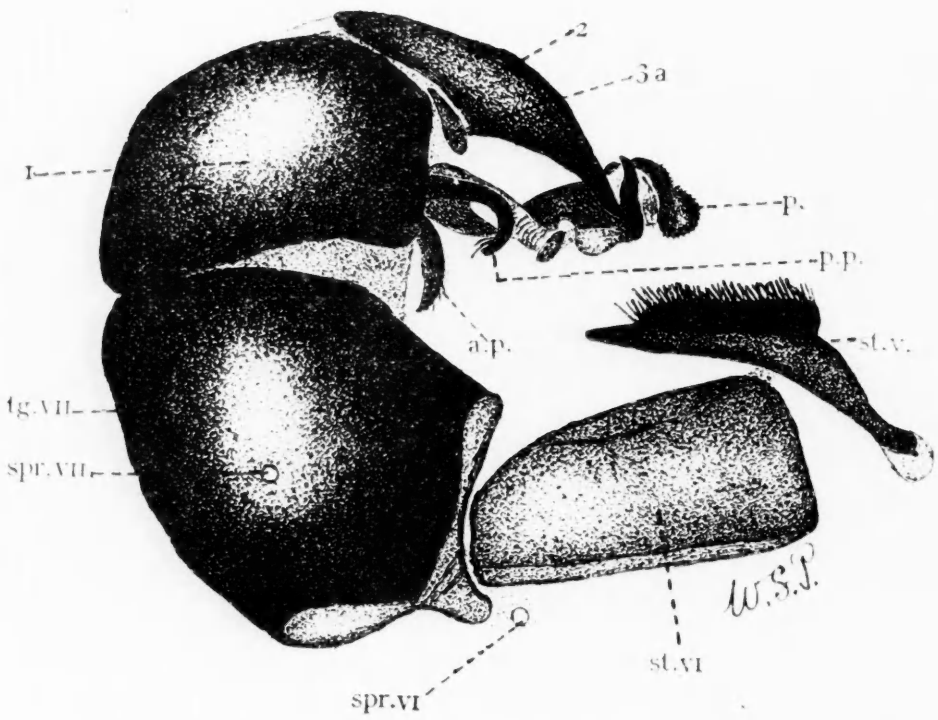

Fig. 4. Terminalia of ♂ *S. crassimargo* in side view. *st. v, st. vi.*—Fifth, sixth sterna ; *spr. vi, spr. vii.*—Sixth, seventh spiracle ; *tg. vi, tg. vii.*—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a.*—Distal segment of ninth coxite ; *a.p.*—Anterior paramere ; *p.*—Phallosome *p.p.*—Posterior paramere (British specimen).

Salient Diagnostic Characters of Male Terminalia. The terminalia in the position during copulation are illustrated in fig. 5, the phallosome lying between the anal cerci. General Characters. Black, not markedly prominent. Sclerites. Sternum 5 (figs. 4 ; 5, *c*) Y-forked, the arms with numerous short and long stiff black hairs on inner surface. Tergum 6 wanting ; sternum 6 (fig. 4) a short, wide, bent plate attached to sternum 5. Tergum 7 a large, long, curved plate. Ninth tergo-sternum (fig. 5, *b*) a short plate with

long posterior arms nearly meeting. Tergum 10 (fig. 4) a long sloping curved plate. APPENDAGES. *Ninth Coxite.* Ventral view. *Distal Segment* as in fig. 5, *d*. Lateral view. Figs. 4 ; 5, *e*. A rather small rounded plate. *Anal Cercus.* Ventral view. Fig. 5, *d*. A short wide plate, anterior end abruptly narrowing to a point. Lateral view as in figs. 4 ; 5, *e*.

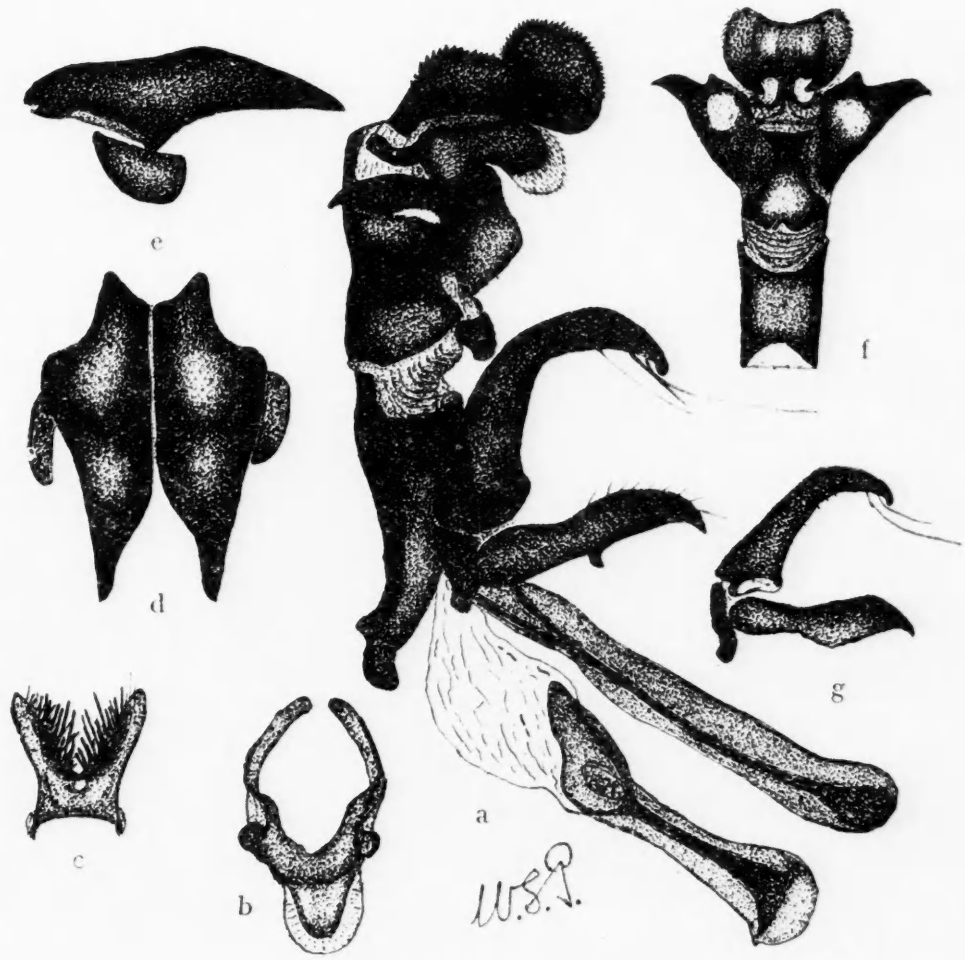

FIG. 5. *a*.—Phallosome and one paramere of *crassimargo* in side view ; *b*.—Ninth tergosternum ; *c*.—Fifth sternum ; *d*.—Ventral view of anal cerci and ninth coxites ; *e*.—Lateral view of anal cercus and distal segment of ninth coxite ; *f*.—Dorsal view of phallosome ; *g*.—Lateral view of right paramere (British specimen).

Phallosome. Lateral view. Fig. 5, *a*. Distal part consisting of a characteristic bilateral, rounded, spined plate and two short pointed wing-like hooks (fig. 5, *f*) ; the proximal part relatively short. *Posterior Process of Phallosome.* Fig. 5, *a*. Projecting backwards in continuation with body of phallosome. *Sperm Pump Sclerite.* As in fig. 5, *a*. *Apodeme of Phallosome.* Fig. 5, *a*. Long and broad. *Parameres.* Lateral view. *Anterior Part.* Fig. 5, *a*, *g*. A rather narrow rod-like plate, ending in a sharp hook, with a row of short hairs on curved surface. *Posterior Part* Fig. 5, *a*, *g*. A long, upstanding, curved rod, the end bent, with one long and two shorter subapical hairs (sometimes only one).

Salient Diagnostic Characters of Female Terminalia. Larvipositor. Segments 6, 7, 9 and 10. General Characters. Black. Fig. 6. The illustration shows the position during copulation. The anal cerci of male (*a.c.m.*) are hooked into the wide genital opening, drawing back tergum 7 and sternum 10 and the chitinous plate of uterus (*sg.*). Sclerites. Tergum 6 consists of two plates joined by membrane (fig. 6, *d*) ; sternum 6 a quadrilateral

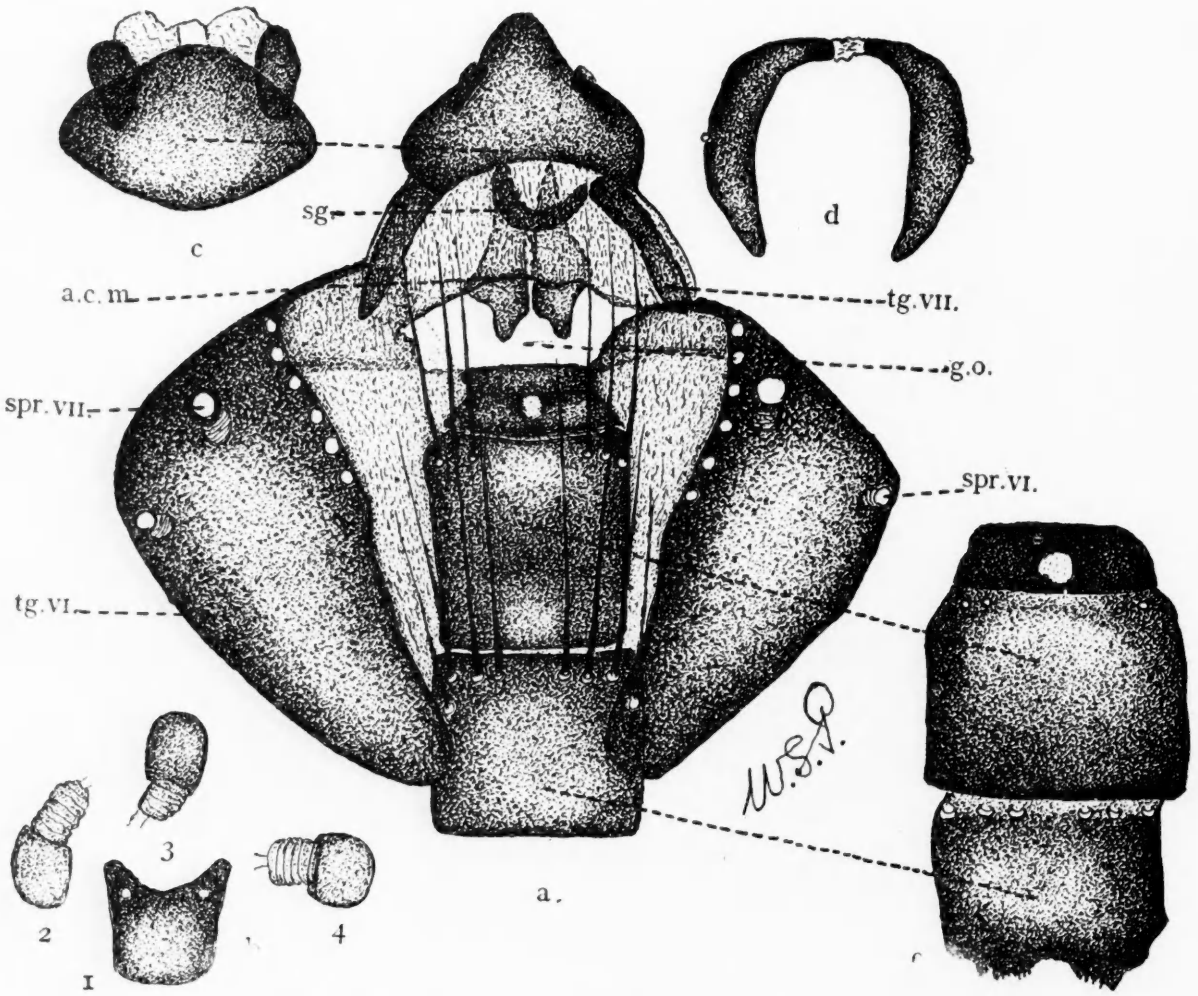

Fig. 6. *a.*—Ventral view of terminalia of ♀ *crassimargo* to show diagnostic characters ; *a.c.m.*—Anal cerci of ♂ hooked into genital opening ; *g.o.*—Genital opening ; *spr. vi, spr. vii.*—Sixth, seventh spiracle ; *sg.*—Signum (chitinous plate of uterus) drawn back by anal cerci of ♂ ; *tg. vi, tg. vii.*—Sixth, seventh terga ; *b.*—1.—Signum, 2, 3, 4, Spermathecae ; *c.*—Tenth sternum and anal cerci ; *d.*—Sixth tergum to show its division into two plates ; *e.*—Sixth and seventh sterna (British specimen).

plate usually with three long, very stout bristles on each side. Tergum 7 two long, rather narrow plates on each side in membrane ; sternum 7 a longer quadrilateral plate with an (apparently) separate distal part, in the centre of which there is a round light area ; we are unable to say definitely whether this is the ninth sternum or part of the seventh. Tergum 9 is wanting. Tergum 10 also wanting ; sternum 10 a large four-sided hairy plate. *Anal Cerci.* Two short folded plates at sides of sternum 10. *Spermathecae.* Fig. 6, *b*, 2, 3, 4. Rather

short. *Chitinous Plate of Uterus* (signum). Fig. 6, *b*, 1. A small plate, forked anteriorly, with a characteristic clear spot on each side. The important diagnostic characters of the female terminalia are the divided 6th tergum and the long seventh sternum with the clear area at the distal end.

Sarcophaga clathrata (Mg.) Böttcher. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF TERMINALIA. ♂. *S. clathrata* is again a medium-sized species like the last, with black terminal segments. It belongs to a very small group of species in which the presutural ac. are well developed, and as it is the only British species showing that character in combination with a narrow frons, which at the vertex is little more than half the width of an eye, it is one of the easier species to identify without examination of the terminalia. It also usually has a single seta or perhaps two longish ones at the base of the first longitudinal vein, which, though slight, seems a good distinguishing character. Moreover, it has a somewhat distinctive appearance, having slate-blue tomentum with strongly developed black markings on thorax and abdomen, those on the latter almost forming three broad continuous lines. *Agnata* Rond. sometimes has presutural ac. developed somewhat, when it might be confused with *clathrata* ; the latter, however, can be recognized by its darker blue-black colour, by the presence of the presutural intra-alar, by the one or two bristles on the base of the first longitudinal vein, and by the well-developed postero-ventral comb on the mid femora—all of which are lacking in *agnata*.

Clathrata has the third and fifth sections of costa about equal ; strong postsutural d.c. ; well-developed marginal setae on second segment ; apical scutellars ; a strong pair of prescutellar ac. ; all sternites with long erect hairs ; no bristles on the seventh tergum ; distinct presutural intra-alars, and its terminal segments altogether smaller in proportion to its size than is usual in the genus. The leg chaetotaxy is as follows :—mid femora with post-ventral series consisting of a few long fine hairs at the base, then before the middle usually two thick longish bristles and then a graduated series to the apex, forming a well-developed comb of short thick thorn-like setae in the apical third. Antero-ventral series irregular, with apical third forming an indefinite comb of not very short fine hairs. Hind femora with complete postero- and antero-ventral series of long hairs, the former longer than width of femora. No striking tibial pilosity, but just a few fine long hairs on apical portion of hind pair on postero- and antero-ventral surfaces.

♀. The ♀♀ can probably be recognized by the same characters as the ♂♂, the colouration and the pattern, presutural ac., and setae on the first longitudinal vein. Its leg chaetotaxy is less well developed.

NOTES. It is probably a common and generally distributed insect, but, whereas most of the species of *Sarcophaga* (sens. lat.) occur during long periods in the summer months, and with perhaps a succession of broods, this seems to have a short spring season and apparently a single brood, as nearly the whole

of a long series in the possession of one of the writers (C.J.W.) was taken in June from only a few localities, chiefly the New Forest, Wyre Forest, Studland and Farley. It seems widely distributed in France and Germany and has been recorded from the Canary Isles, Southern Sweden and Finland. It has been reported as being bred from egg masses of *Epeira cornuta* Koch., and also from the locusts *Acridium aegyptium* L. and *Stauronotus maroccanus* Thunb., but these latter records seem to require confirmation. It may prove to be a true parasite and not a mere scavenger like most of the species of the genus, but information is lacking.

Enderlein says that our species is not the true *clathrata* of Meigen, and has renamed it *Böttcheri*; but since Villeneuve has already given this name to another species it cannot be used for this one; we hesitate to re-name it without more positive information.

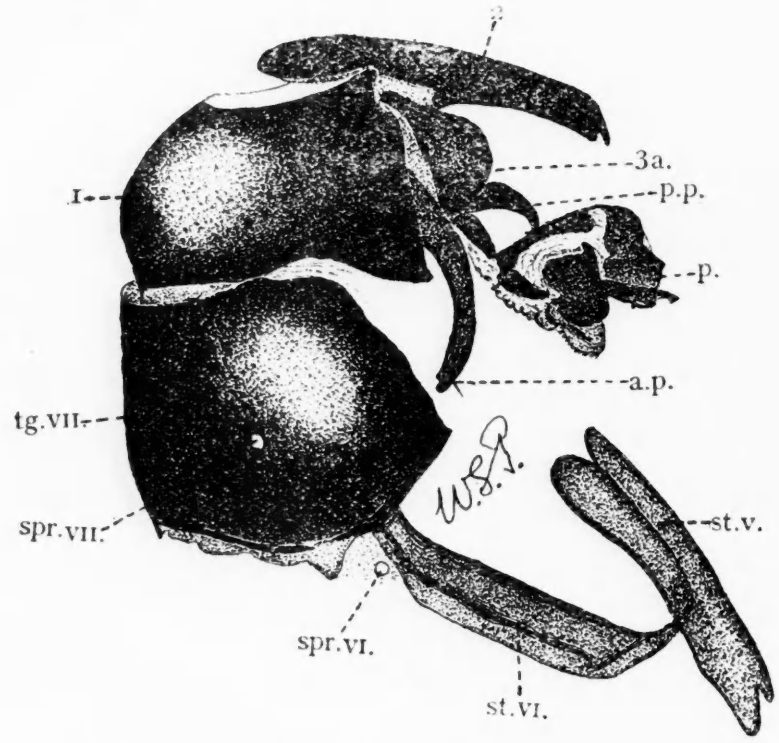

FIG. 7. Terminalia of ♂ *S. clathrata* in side view. *st.v, st. vi.*—Fifth, sixth sterna; *spr. vi, spr. vii.*—Sixth, seventh spiracle; *tg. vi, tg. vii.*—Sixth, seventh terga; 1.—Tenth tergum; 2.—Anal cercus; 3*a.*—Distal segment of ninth coxite; *a.p.*—Anterior paramere; *p.*—Phallosome; *p.p.*—Posterior paramere (British specimen).

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. The terminalia are illustrated in side view in fig. 7. GENERAL CHARACTERS. Black not markedly prominent. SCLERITES. Sternum 5 (figs. 7; 8, *c*) Y-forked, without any characteristic hairing or bristling. Tergum 6 wanting; sternum 6 (fig. 7) a long, narrow, curved plate. Tergum 7 (fig. 7) a short curved plate. Ninth tergo-sternum (fig. 8, *b*) small, with posterior processes joined. Tergum 10 (fig. 7) a short wide curved plate. APPENDAGES. *Ninth Coxite.* Ventral view. *Distal Segment.* As shown in fig. 8, *d*. Lateral view. Fig. 8, *f*. A rather

large triangular plate, the posterior end pointed. *Anal Cercus.* Ventral view. As shown in fig. 8, *d*. Lateral view. Fig. 8, *f*. A short, stout, rod-like plate ending abruptly in a sharp point with a notch (very characteristic).

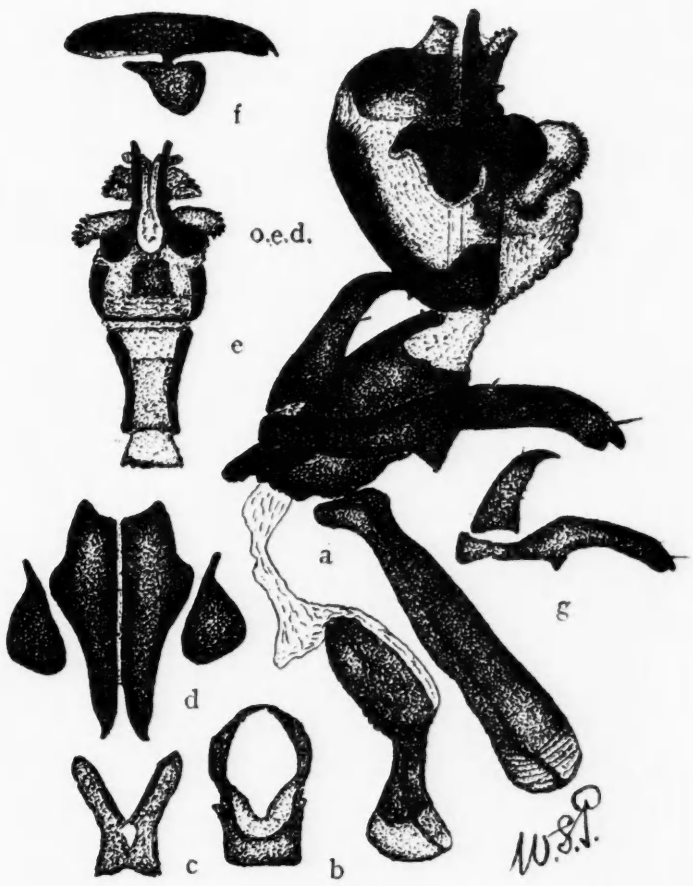

FIG. 8. *a*.—Phallosome and one paramere of *clathrata* in side view ; *b*.—Ninth tergo-sternum ; *c*.—Fifth sternum ; *d*.—Ventral view of anal cerci and distal segments of ninth coxites ; *e*.—Dorsal view of phallosome to show opening of ejaculatory duct in the middle between the two rods ; *f*.—Lateral view of anal cercus and distal segment of ninth coxite ; *g*.—Lateral view of right paramere (British specimen).

Phallosome. Lateral view. Fig. 8, *a*. Short, the distal end bulbous, with two pairs of spined plates and an admedian pair of rods, between which the ejaculatory duct opens (fig. 8, *e*) ; proximal part long and wide. *Posterior Process of Phallosome.* Fig, 8, *a*. Short. *Sperm Pump Sclerite.* Fig. 8, *a*. Large with long handle. *Apodeme of Phallosome.* Fig. 8, *a*. Long and wide. *Parameres.* Lateral view. Anterior Part. Fig. 8, *a*, *g*. A long curved plate, expanded flange-like at end with a notch. *Posterior Part.* Fig. 8, *a*, *g*. A much smaller, upstanding, pointed rod, usually with a small hair about middle.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. LARVIPOSITOR. SEGMENTS 6, 7, 9 and 10. GENERAL CHARACTERS. Black, tergum 6 with a continuous row of stout bristles along its inner margin. SCLERITES. Fig. 9. Tergum 6 consisting of two separate plates joined by membrane ; sternum 6

as in fig. 9, *b*. Tergum 7 a pair of long narrow plates lying on each side of sternum 10 in membrane ; sternum 7 as in fig. 9, *b*, and, apparently, consisting of two parts, the proximal more heavily chitinized than the distal. Tergum and sternum 9 wanting. Tergum 10 wanting ; sternum 10 a triangular plate. *Anal Cerci.* Small folded plates. *Spermathecae.* As in fig. 9, *d*. *Chitinous Plate of*

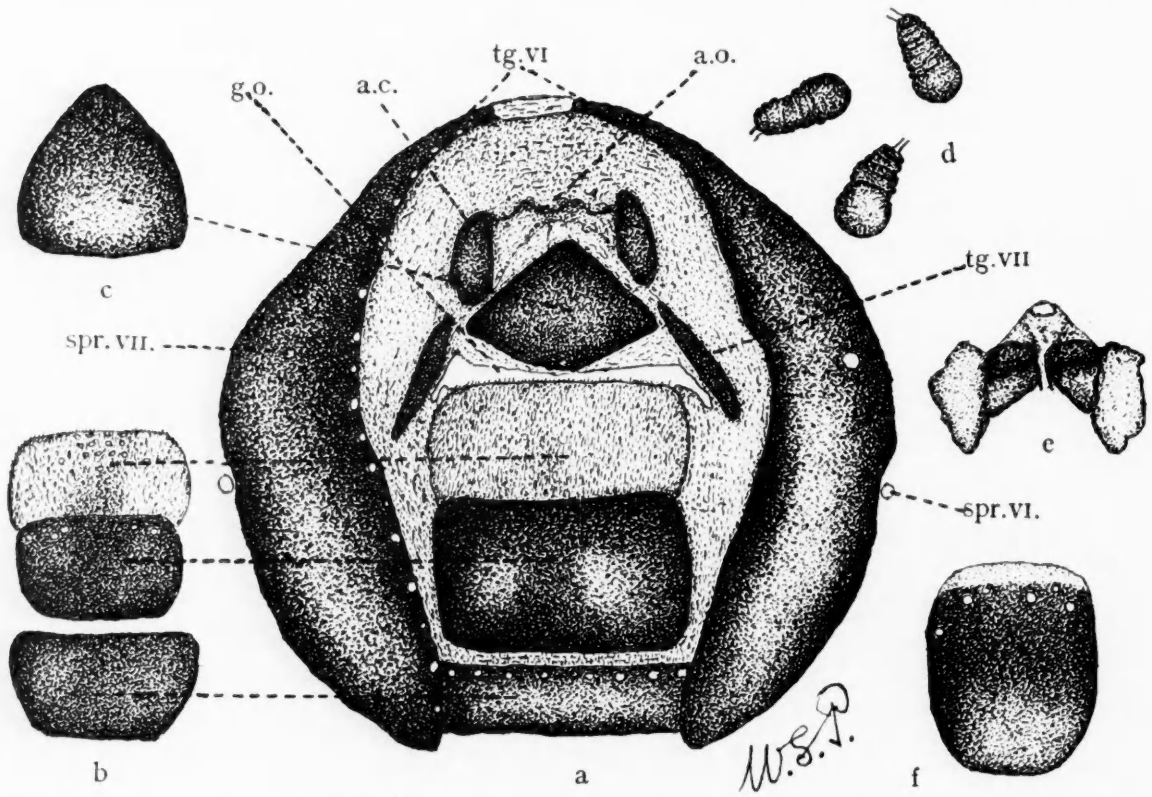

FIG. 9. *a*.—Ventral view of terminalia of ♀ *clathrata* to show diagnostic characters ; *a.c.*—Anal cerci ; *a.o.*—Anal opening ; *g.o.*—Genital opening ; *spr. vi, spr. vii.*—Sixth, seventh spiracle ; *tg. vi, tg. vii.*—Sixth, seventh terga ; *b*.—Sixth and seventh sterna ; *c*.—Tenth sternum ; *d*.—Spermathecae ; *e*.—Signum ; *f*.—Fifth sternum (British specimen).

Uterus (signum). Small and of a characteristic shape, as shown in fig. 9, *e*. The important diagnostic characters of the ♀ terminalia are the divided 6th tergum and the characteristic shape of tergum and sternum 7.

Sarcophaga dissimilis Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. *S. dissimilis* is a medium-sized to small species, usually 5–6 mm. long. It belongs to a small group of species easily recognized by the presence of a row of setae on the first longitudinal vein. It can be distinguished from *setipennis* Rond. by its narrow frons of about half eye width (though this varies somewhat), that of *setipennis* being nearly of eye width, but *offuscata* Sch., the only other British species with which it may be confused, is not so easily separated without examination of the terminalia. *Dissimilis* tends to an extension of the shiny black markings of the abdomen, though this is not so noticeable in the ♂♂ as in the ♀♀ ; in the former the dark markings coalesce and form something like a hind marginal band, and the general effect is more

shining than is usual in *Sarcophaga* ; in *offuscata* the grey tomentum is well developed and the dark markings remain longitudinal and distinct. *Dissimilis* has strong apical scutellars and a distinct row of hind marginal bristles on the seventh tergum ; in *offuscata* neither is so well developed.

It has three post-sutural d.c. ; a pair of prescutellar ac., but none before the suture, though the space between the d.c. is clothed with rather long stiff hairs ; it has strong hind marginals on the second segment, and a long strong costal thorn. The terminal segments are black and shining, with no tomentum. Leg chaetotaxy as follows :—front femora with the usual postero-ventral row of long bristle-like hairs complete ; dorsal and postero-dorsal rows shorter and less developed but also complete ; mid femora with a complete row of postero-ventral long bristle-like hairs, not becoming shorter and thicker towards apex, and, therefore, forming no comb ; antero-ventral bristles with a row of short comb-like apicals, but not strongly developed, and usually four long, strong bristles about the middle ; hind femora with complete antero- and postero-ventral rows of bristle-like hairs, which, however, do not exceed in length the width of femora ; also numerous irregular stronger bristles on the antero-dorsal aspect, tending to run in two rows ; all the tibiae free from pilosity, excepting for a row of about eight fine hairs behind apex of hind pair.

♀. In this species the ♀♀ are more easily recognized than the ♂♂, as apart from the general characters, and in particular the setae on the first longitudinal vein, the extreme reduction of the abdominal tomentum is very characteristic. There is some variation in this respect, but as a rule there are only small white triangular spots at the fore corners of the second and third segments, and some indications of the usual pattern on the fourth ; the abdomen too is broader than usual and the general effect very shining. It is so different in its appearance from any other species of the genus that Brauer and von Bergenstamm failed to associate it with the ♂, and erected a separate genus for it, calling it *Heteronychia chaetoneura*, which name is to be found in the Verrall list.

Notes. It is one of our more abundant species and probably occurs everywhere over a long season, but it is especially to be found on foliage in spring, when we have often seen it in some numbers. It is recorded as being widely distributed in Germany, France and Italy, etc., but Böttcher says that it is not common in Germany, and Lundbeck does not include it among the Diptera of Denmark. Nothing seems to have been recorded about its life-history, but from its mode of occurrence on foliage we have suspected that it may prove to be a true parasite.

Salient Diagnostic Characters of Male Terminalia. The terminalia as seen in side view are illustrated in fig. 10. General Characters. Black and not prominent. Sclerites. Fig. 10. Sternum 5 (figs. 10 ; 11) Y-forked almost to base, arms with numerous short stiff bristles. Tergum 6 wanting ; sternum 6 (fig. 10) a long, wide, curved plate. Tergum 7 (fig. 10) a long, rather narrow, curved plate. Ninth tergo-sternum (fig. 11, *f*) a long narrow plate, the

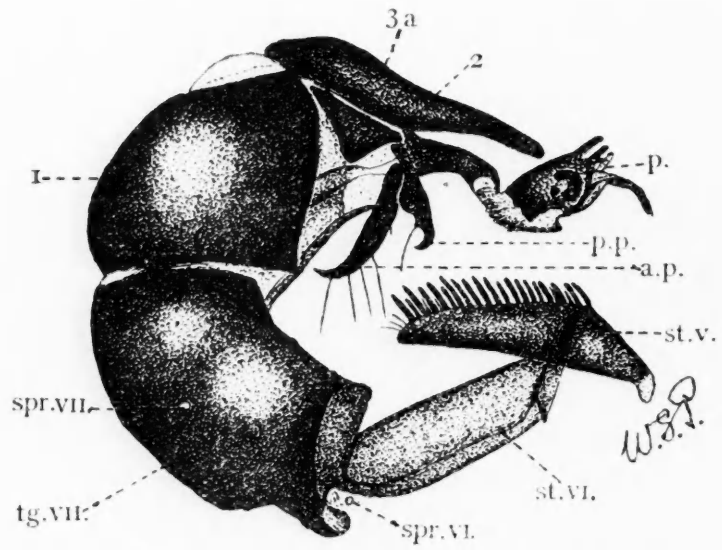

FIG. 10.—Terminalia of ♂ *S. dissimilis* in side view. *st. v, st. vi.*—Fifth, sixth sterna ; *spr. vi, spr. vii.*—Sixth, seventh spiracle ; *tg. vi, tg. vii.*—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a*.—Distal segment of ninth coxite ; *a.p.*—Anterior paramere ; *p.*—Phallosome ; *p.p.*—Posterior paramere (British specimen).

posterior processes not meeting. Tergum 10 (fig. 10) a short curved plate. APPENDAGES. *Ninth Coxite.* Ventral view. *Distal Segment.* As in fig. 11, *c* ; note the sclerosed 8th spiracle. Lateral view. Fig. 11, *d*. A small triangular plate. *Anal Cercus.* Ventral view. Fig. 11, *c*. A long bluntly pointed rod, the anterior three-quarters free.

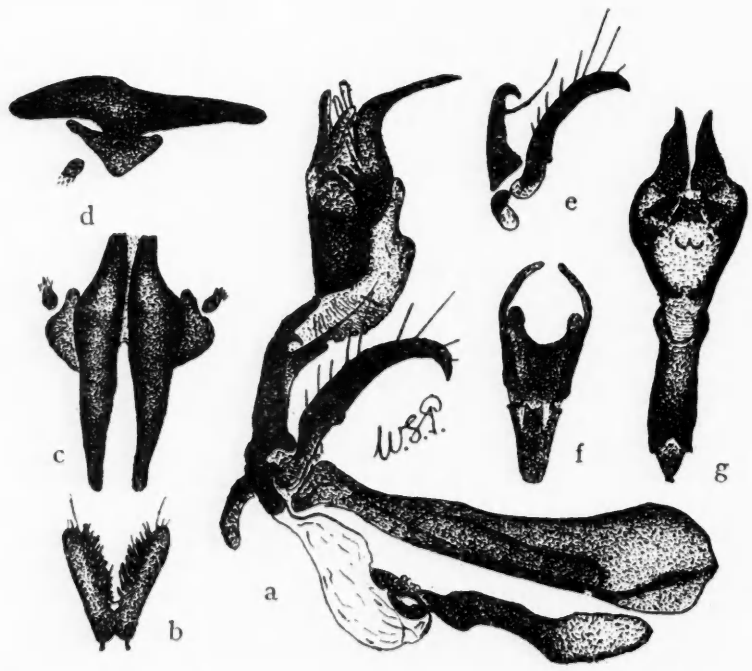

FIG. 11. *a.*—Phallosome and one paramere of *dissimilis* in side view ; *b.*—Fifth sternum ; *c.*—Ventral view of anal cerci and distal segments of ninth coxites ; note sclerosed eighth spiracle ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Lateral view of right paramere showing two parts ; *f.*—Ninth tergo-sternum ; *g.*—Dorsal view of phallosome (British specimen).

Phallosome. Lateral view. Fig. 11, *a*. Rather slender but long, with a very characteristic long chitinous rod on each side of distal end pointing upwards and forwards ; dorsal (front) view as in fig. 11, *g*. *Posterior Process of Phallosome*. Fig. 11, *a*. Long and bent. *Sperm Pump Sclerite*. Fig. 11, *a*. Long. *Apodeme of Phallosome*. Fig. 11, *a*. Very long and wide. *Parameres*. Lateral view. *Anterior Part*. Fig. 11, *a*, *e*. Long curved rod with a hooked blunt point and a row of long hairs along curved border. *Posterior Part*. Fig. 11, *a*, *g*. A much shorter hooked rod, with one long curved subapical hair.

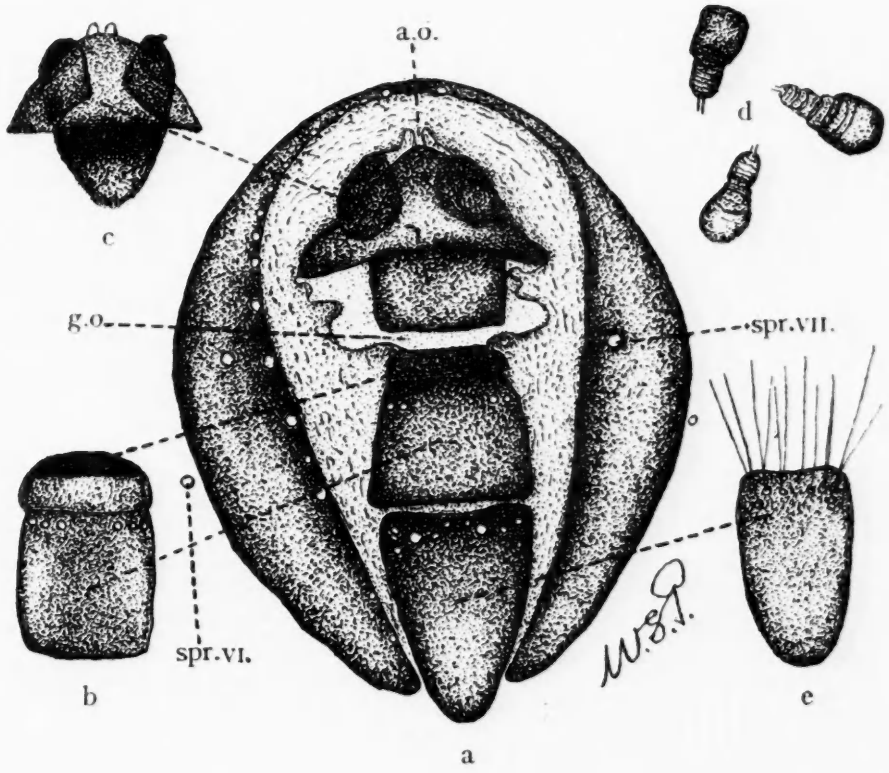

FIG. 12. *a*.—Ventral view of ♀ terminalia of *dissimilis* to show diagnostic characters ; *a.o.*—Anal opening ; *g.o.*—Genital opening ; *b*.—Seventh sternum ; *c*.—Ninth and tenth sterna and anal cerci ; *d*.—Spermathecae ; *e*.—Sixth sternum (British specimen).

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. LARVIPOSITOR. SEGMENTS 6, 7, 9 and 10. GENERAL CHARACTERS. Black and surrounded by a continuous row of stout bristles along inner margin of tergum 6. Fig. 12. Tergum 6 (fig. 12) consisting of a single plate ; sternum 6 as in fig. 12, *e*. Tergum 7 wanting ; sternum 7 (fig. 12, *b*) a small quadrilateral plate with an apparently distinct distal part ; this cannot be sternum 9, as that plate is present in its normal position. Tergum 9 wanting ; sternum 9 (fig. 12, *c*) a small triangular plate forming the posterior wall of genital opening. Tergum 10 wanting ; sternum 10

as in fig. 12, *c*. *Anal Cerci*. Rather large curved plates. *Spermathecae*. As in fig. 12, *d*. *Chitinous Plate of Uterus*. Wanting. The important diagnostic characters of the ♀ terminalia are the single sixth tergum, the characteristic seventh sternum, and the well-marked (apparent) ninth sternum which can usually be made out by hooking back tergum 10 with a blunt hook in genital opening.

(*To be continued*)

TWO NEW ANOPHELINES FROM SOUTH AFRICA

BY

B. DE MEILLON, D.Sc.

(*South African Institute for Medical Research, Johannesburg*)

AND

A. M. EVANS, D.Sc.

(*Department of Entomology, Liverpool School of Tropical Medicine*)

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Anopheles (Myzomyia) cameroni sp.nov.

This species is closely related to *A. rhodesiensis* Theo., from which it differs strikingly in possessing well-developed pale spots on the wing field. It is rather larger than *rhodesiensis*, and on the mesonotum true scales occur more than a quarter of the distance from the anterior border.

Only one specimen, a ♀, is known so far.

ADULT. ♀. *Head* (fig. 1, B). Upright forked scales, especially at sides of head, unusually long and narrow, as in *A. rhodesiensis* ; frontal tuft reduced as in that species. Antenna with pale scales on first flagellar segment. Palpal index 0·33. Palp with 3 pale bands, the proximal and middle bands involving both ends of the articulations of the second and third and the third and fourth segments respectively ; the apical band occupying the whole of the last segment.

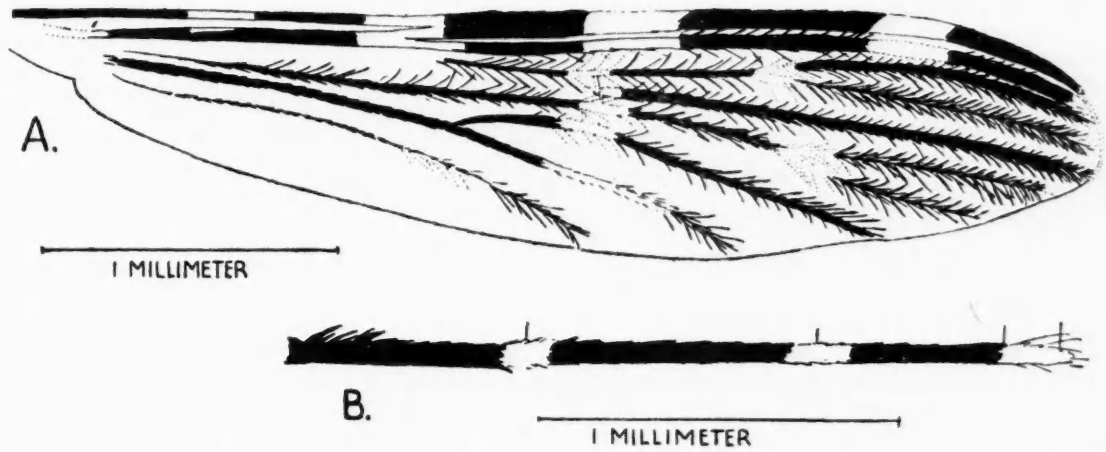

FIG. 1. *Anopheles cameroni* sp. n.: A.—Right wing of type ♀, drawn with camera lucida but diagrammatic to the extent that some dark scales have been added to the partly denuded portions of certain veins, especially the branches of the fourth ; fringe omitted ; B.—Palp ; ends of segments indicated by short lines. (Drawn by A. M. E.)

Pharynx. Pharyngeal armature of *Neomyzomyia* type. Teeth 9 in number and very similar in structure to those of *rhodesiensis*, the filament being relatively broad with fimbriated apex. The filaments appear to be somewhat longer than in *rhodesiensis*, and the transparent areas enclosed by the basal bulla are less

conspicuous and more basally situated than in similarly orientated preparations of that species. The post-armature membrane is markedly striated, but, as in some specimens of *rhodesiensis* examined, does not form definite ridges.

Thorax. Integument very similar to that of pale specimens of *rhodesiensis*. Mesonotum mainly clothed with dark bristles and short hairs and hair-like scales, but whitish, narrow, curved scales extend back from the anterior promontory for about 2/7 the length of the mesonotum. Pleural chaetotaxy much as in *rhodesiensis*.

Abdomen without scales, even on cerci.

Legs. Fore and mid legs dark scaled with small pale spots at apices of tibiae. Hind legs also dark scaled, as in *rhodesiensis*.

Wings (fig. 1, A). Right wing as shown in the illustration. Pale scales in costal region with yellowish tinge. Markings differing from those of *rhodesiensis* mainly as follows. Costa with pale area in basal quarter, the area extending on to the subcosta. Wing field with well-marked pale spots present in the following positions :—at bases of fork cells ; near cross-veins on stems of second and fourth, base of third and upper branch of fifth veins ; at apices of first and branches of second ; on lower branch of fifth vein (large area). Sixth vein with an ill-defined area of obscurely pale scales on outer half. Apex of wing denuded, but membrane at border pale from above end of first vein to end of third. Fringe largely denuded on outer half. Outstanding scales long and narrow. The left wing is damaged, but, as far as can be seen, the markings are similar to those of the right.

Wing length. 3·7 mm.

SOUTH AFRICA : Cape Province ; type ♀, Fishhoek, near Cape Town, January, 1934, collected by one of the writers (B. De Meillon).

Type temporarily in Liverpool School of Tropical Medicine.

This insect is named in honour of Mr. W. A. J. Cameron, the first secretary of the South African Anti-Malarial Association founded in 1910, who, as manager and secretary of the South African Institute for Medical Research, has been responsible for much of the progress of the study of the entomology of malaria in South Africa.

A. cameroni bears a superficial resemblance to *A. sergenti* Theo., but the structure of the pharyngeal armature and the shape of the head scales show that there is no close affinity between the species. Christophers and Puri (1931), in their very valuable account of *A. rhodesiensis*, described for the first time many of its distinctive characters, such as the long narrow head scales and the *Neomyzomyia*-like pharyngeal armature. It should be noted, however, that in many specimens examined by the present writers the palpal bands of the ♀ are of equal distinctness, and the apical band usually involves at least the whole of the last segment. Further, the first flagellar segment of the antenna usually bears scales which, though commonly dark, may be pale, as in the present species.

Anopheles walravensi var. **milesi** var.nov.

This variety, of which only one specimen is known, differs from the type form as follows :—

ADULT. ♀. *Wings* (fig. 2, A). Wing field with distribution of dark scales unique ; the veins almost entirely pale scaled, except towards the apices of the branches of the second, third, upper branch of fourth and lower branch of fifth veins, where dark areas of moderate size occur ; elsewhere there are only a few minute dark spots. Basal portion of fringe pale scaled, as in specimens of *distinctus*. The right wing of the type is illustrated, and both wings show the striking dark band across the apex, formed by the concentration of dark scales at the distal ends of the veins. The left wing differs from the right mainly in the absence of a pale subcostal spot on the first vein and in the presence of an additional dark spot on the stem of the second vein.

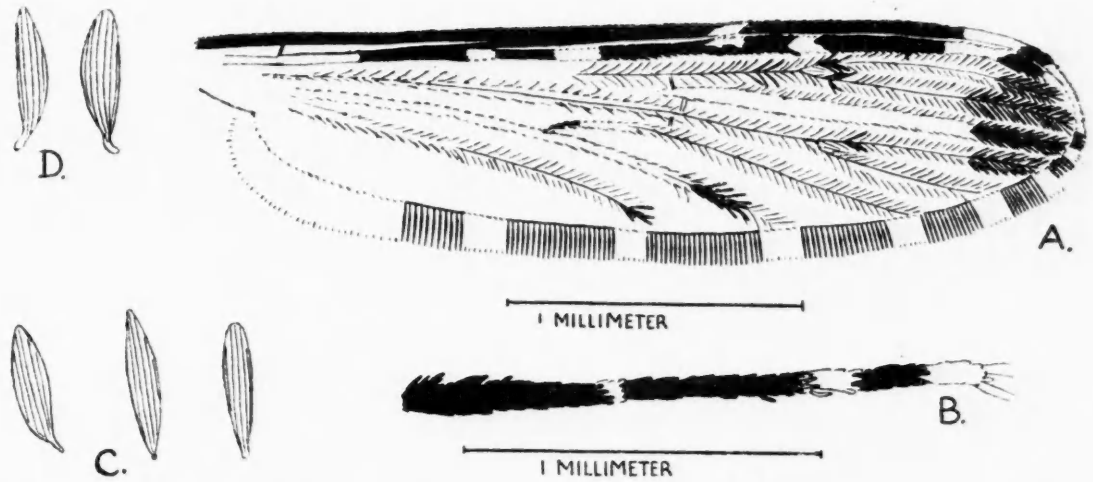

FIG. 2. *Anopheles walravensi* var. *milesi* var. n. : A.—Right wing of type ♀ ; B.—Palp ; C.—Scales from median area of mesonotum ; D.—The same of *A. squamosus* Theo. (A. and B. drawn by A. M. E. ; C. and D. by B. De M.)

In the limited number of specimens of the type form of *walravensi* known the markings of the wing field are not abnormal ; the dark areas are shorter on the apical portions of some of the veins which reach the apex of the wing, and elsewhere there are dark areas of considerable extent. In other respects *milesi* resembles *walravensi*, the markings of the palps, of the legs and of the costa and first vein, as well as the scaling of the mesonotum, being similar to those of a specimen of the type form from Ndola, Northern Rhodesia, which was determined by Dr. Edwards. It is possible that, when the morphological characters can be compared, specific differences from *walravensi* may be found. In view, however, of the amount of variation exhibited by the wing markings of *distinctus* and other species apparently allied to *walravensi* (see Evans, 1934), it seems best, for the present, to regard *milesi* as a variety of this latter species.

SOUTHERN RHODESIA : Victoria Falls ; type ♀ collected, August 13th, by Mr. Victor Miles.

Type ♀ in Liverpool School of Tropical Medicine. The variety is named in honour of its discoverer.

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THE RÔLE OF THE NATURAL DEFENCE FORCES IN THE EVOLUTION OF THE DRUG-RESISTANCE OF TRYPANOSOMES

II.—THE RAPID PRODUCTION OF GERMANIN-FAST *T. BRUCEI* STRAINS IN ANIMALS WITH PARALYZED DEFENCE

BY

NIKOLAUS VON JANCSÓ

AND

HERTHA VON JANCSÓ

(*Chemotherapy Department, Pharmacology Institute, Royal Hungarian University, Szeged*)

(*Received for publication* 23 *June*, 1934)

A considerable advance was made in knowledge of chemotherapy when Yorke, Murgatroyd and Hawking (1931) and, simultaneously but independently, Jancsó (1931, 1932, 1933) investigated by various methods the mechanism of the production of drug-resistance in trypanosomes.

Yorke and his co-workers kept both normal and atoxyl-fast trypanosomes for one hour at 37° C. in a nutrient fluid containing reduced tryparsamide thioglycollate, and found that the strains behaved differently, in that the normal strain absorbed the drug quickly, whilst the atoxyl-fast strain absorbed an almost inappreciable amount.

We have ourselves studied the mechanism of drug-resistance by means of certain new methods whereby chemotherapeutic substances are revealed in the bodies of the trypanosomes. In trypanosomes recovered from treated animals we have demonstrated, firstly, the presence of chemotherapeutic substances, secondly, the amount of chemotherapeutic substance vitally fixed by the parasites at various intervals after treatment, and thirdly, the localization of the drug fixed in the flagellate bodies.

The first method is based on our observation that trypanosomes are photo-sensitized after the treatment of the host with certain chemotherapeutic substances (Jancsó, 1931*a*). In the case of trypaflavine, flavicide and other efficient derivatives of acridine, and in that of the styryl-quinoline compounds, this photo-sensitivity is so high that the causal organisms cease to move in the light of the dark-field microscope, and photo-dynamic death takes place. By setting up a standard illumination and by measuring the photo-sensitivity, we succeeded in determining the relative drug-content of individual trypanosomes (Jancsó, 1931*a*, *b*, *c*). By our second method, which furnishes quantitative results (Jancsó, 1932*a*, *b*), a large number of trypanosomes is isolated from the blood of animals treated with various dye-like chemotherapeutic substances, and the amount of the dye they contain is determined colorimetrically after extraction by suitable solvents. Finally, by using fluorescent drugs and by illuminating a fresh specimen or a dry smear made

from the blood of a treated mouse or rat infected with trypanosomes, we succeeded in revealing the chemotherapeutic substances stored up in the trypanosomes. The illumination was carried out in a fluorescent microscope with invisible ultra-violet rays, the wave length ranging from 2,000 to 3,000 A°. The various fluorescent colours of the dyes are clearly seen at the places where they are stored within the body of the flagellates (Jancsó, 1932*b* ; N. and H. von Jancsó, 1933 ; cf. also Fischl and Schwenk, 1932).

These methods have proved very successful, especially with various acridine and styryl-quinoline compounds ; the optimally efficient compounds—trypaflavine and 2-p-acetyl-aminostyryl-6-dimethylamino-quinoline methosulphate*—were found to be particularly suitable. By all three methods, drug-sensitive strains of *Trypanosoma brucei* or *T. gambiense* were shown to absorb to a striking extent the efficient acridine and styryl-quinoline compounds in the blood of the treated animal. Thus a very appreciable amount of the drugs given enters the bodies of the trypanosomes—and this explains their specific chemotherapeutic action. In striking contrast, only traces of the chemotherapeutic substance could be found in drug-resistant trypanosomes which had received a similar treatment. The ratio of the amounts of the drug absorbed by normal and resistant strains was often more than 30 : 1, whether resistance had been obtained directly by systematic treatment with trypaflavine, or indirectly by rendering the strain resistant to arsenicals (Jancsó, 1931*c*, 1932*a*, *b*, *c* ; N. and H. von Jancsó, 1932, 1933). The characteristic behaviour of the resistant trypanosomes manifests itself in the photo-dynamic experiments by a relatively slight sensitivity to light ; and in the ultraviolet-ray experiments by the fact that the drug-resistant flagellates, unlike the normal ones, remain invisible, or exhibit only feeble fluorescence at certain points, such as the blepharoplast (Jancsó, 1932*c*). In these cases, drug-fastness is therefore due to a specific impermeability of the parasite body. Our researches with normal strains have proved that acridine, styryl-quinoline and nitroacridine compounds, as well as pyronine and tryparosan, are vitally fixed, partly by the protoplasm, but chiefly by the nucleus and blepharoplast (Jancsó, 1932*c*), like the nucleotropic dye-stuffs in stained preparations.

Since the compounds can actually be seen in the cells, several interesting observations on the impermeability of drug-resistant trypanosomes, which have hitherto been inexplicable, can now be easily understood. We observed the phenomenon, first described by Werbitzki (1910), of the loss of the staining-capacity of the blepharoplast after subtherapeutic doses of several of the compounds, and the origin of so-called blepharoplastless strains of trypanosomes. Our researches with the microfluoroscopical method have shown that drugs endowed with this property—such as trypaflavine, flavicide or tryparosan—are abundantly stored in the blepharoplast ; this fact suggests that their deleterious action may be greatest on the blepharoplast, although the nucleus, where we have found trypaflavine also located in considerable quantities, may be similarly affected. That such a disappearance of the blepharoplast in strains made resistant to arsenicals does not occur

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*The *chloride* corresponding to this preparation was described by Browning, Cohen, Ellingworth and Gulbransen (1929) as ' preparation 90.' According to these authors, it is the most efficient of the styryl derivatives. In nagana of mice its chemotherapeutic index is as high as 1 : 50, and nagana-infected rabbits are curable by a single dose.

even after many months' treatment (Leupold, 1925) is due to the fact that these arsenic-fast trypanosomes were impermeable to trypaflavine, and consequently the acridine dye did not come into contact with the cell in a concentration sufficient to destroy it (Jancsó, 1932*c*).

By the aid of the same method we also succeeded in demonstrating that therapeutic interference is a phenomenon which is closely allied to drug-fastness. The classical example of therapeutic interference is that first described by Browning and Gulbransen (1922), in which the curative action of trypaflavine on trypanosomes was suppressed by a previous treatment with subtherapeutic doses of parafuchsin. We have proved that parafuchsin renders the trypanosomes for some time impermeable to trypaflavine, thus preventing therapeutic action (Jancsó, 1931*b*, 1932*c*).

These researches have considerably increased knowledge of the chemotherapeutic mechanism of a number of important chemotherapeutic agents, and at first sight may give the impression that the theoretical analysis of the phenomenon of drug-fastness is now complete. However, the question whether these results permit of generalizations cannot immediately be answered in the affirmative, as there is the possibility, or even the probability, that drug-resistance may in certain cases be caused by another mechanism. We may, however, assume that in drug-fastness there is always an adjustment of the parasites to the specific action of the chemotherapeutic agent.

The present paper describes experiments with germanin (' Bayer 205 '). Unpublished work indicates that Bayer-fastness presents certain features which cannot be explained on the mechanism discussed above.

These researches are concerned with the behaviour of a *Trypanosoma brucei* strain originally made fast both to organic arsenicals and to arsenious acid. The strain was subsequently also made germanin-fast by systematic treatment in mouse passages ; the curative dose of the normal strain was 0·1 mgm. of germanin per 20 gm. body weight, but 5 mgm. failed to cure mice infected with the resistant strain. During the process of making the trypanosomes germanin-fast, the reactions *in vitro* to germanin were repeatedly tested. The examination was made by means of a method devised by us whereby the trypanocidal action *in vitro* of germanin on *T. brucei* is demonstrated, and the generally accepted view that germanin is ineffective outside the body is disproved (N. and H. von Jancsó, 1934). The minimal lethal concentration for the original strain was 1 in 50,000 germanin in the nutrient fluid. Contrary to what we had expected, the sensibility of the strain to germanin *in vitro* did not decrease during the process of making it germanin-fast ; in eight of the experiments made at different stages during the development of resistance, the strain was killed by the 1 in 50,000 concentration of germanin—and killed even more quickly than the original strain. In the last two *in vitro* experiments, in which drug-fastness had reached the maximum, both the original and the drug-fast strains behaved in the same way. Thus, a germanin-fast strain behaves quite differently from, say, a trypaflavine-fast strain whose resistance to the acridine compound persists *in vitro*. However, when the trypanosomes, after being separated from the germanin solution by centrifugation and subsequently washed, were inoculated into mice either intravenously or subcutaneously, the drug-fast and the parent strains immediately showed different behaviour. In the case of the parent strain it was found that trypanosomes which had been in contact with germanin 1 : 10,000 for 14 hours, or 1 : 50,000 for rather longer, always failed to infect mice ; but in that of the resistant strain contact with germanin 1 : 10,000 for 28 hours or 1 : 50,000 for 45 hours failed to render the trypanosomes non-infective for mice.

Hence, in this case, too, drug-fastness proved to depend largely upon the habituation of the parasites to the chemotherapeutic substance ; in the course of the production of drug-resistance the trypanosomes gradually acquire the ability to withstand the specific action of the germanin. At the present stage of our experiments, it is quite impossible to give an adequate explanation for the interesting fact that drug-fastness does not reveal itself *in vitro*, and that the strain is, at least at the initial stages, more easily killed by the relatively high dilution of a 1 in 50,000 concentration of germanin than the parent strain. Consequently, we can no longer accept the view that toxic action *in vitro* is non-specific unless the trypanosomes show specific drug-fastness. The experiment described is, moreover, a valuable proof that the trypanosome is the point of attack of the germanin, and that the specific action is due to the germanin itself, and not to a reaction product.

No less important than the elucidation of the problem of drug-resistance to germanin is investigation into solganal-fast strains of *Spirochaeta recurrentis* (cf. Feldt, 1932). As we have pointed out elsewhere (N. and H. von Jancsó, 1934), a study of the mechanism of action of solganal will probably lead to the solution of several fundamental questions of chemotherapy. We suggest that this drug exerts its specific action by affecting the surface of the spirochaete body. In its curative action, an opsonin-like mechanism plays an important rôle (N. and H. von Jancsó, 1934), though, of course, in such a mechanism drug-resistance cannot be due to the impermeability of the protoplasm, since the drug does not invade it.

As these examples show, we are, in spite of considerable advances, still far from being able to give a definite idea of the mechanism of drug-resistance if we consider merely two factors—the chemotherapeutic substance and the parasite. The experiments described in this paper show that there is yet a third factor which plays an important part in the genesis of drug-fast trypanosomal strains, namely, the natural protective power of the host. The process of production of drug-resistant strains exerts a particular influence on the protective power of the host, the existence of which has not so far been taken into consideration. The importance of this factor is due to the fact that it alone guarantees success in the chemotherapy of trypanosome-infection in certain cases ; we shall show, for instance, that the abolition of these functions in infected rats or mice renders what is a normally curative course of germanin ineffective.

EXPERIMENTS

The *Trypanosoma brucei* strains used for the experiments were as follows :

1. For the experiments in Table I and fig. 1, Prowazek strains.
2. For the experiment in Table I, ser. I, a trypaflavine-fast strain cultured from the above Prowazek strain.

3. For the experiments on rats (Table II), the above-mentioned Prowazek strain made resistant to all arsenicals.

The germanin dose is indicated in grams per 20 gm. body weight : hence, 1/1,000 means 0·001 gm. per 20 gm. subcutaneously.

The elimination of the biological defence mechanism in mice was effected by splenectomy 2 to 4 hours before treatment, followed by intravenous treatment with electro-colloidal copper 3 or 4 hours after the injection of germanin ; for this purpose 0·1 c.cm. per 20 gm. body weight of the copper solution, diluted with 4 parts of distilled water, was given.

The general plan of the experiments in Table I, series B-H, was as follows. It was first sought to learn the behaviour of trypanosomes in normal mice and in mice which had been splenectomized and treated with copper, treated with a subtherapeutic (relapse) dose of germanin, and again treated on the occasion of each relapse. In the normal animals, a series of doses of 1 in 35,000 effected a permanent cure. In the prepared mice, however, the strain quickly became resistant to the dose and was transferred to a fresh group of mice which were treated with ever-increasing doses. In order to approximate to the conditions of treatment of first passage animals, for the normal mice we chose a dose sufficient to cause the disappearance of the causative agents ; in the prepared animals, however, the same dose only produced a transient diminution of the number of trypanosomes, without causing them to disappear from the blood. In spite of the fact that drug-fastness continued to increase, it was not difficult to ascertain the requisite dose, since the increase in drug-resistance was fairly regular and approximated to a geometrical progression (cf. below). In order to demonstrate the pronounced effect of the exclusion of the defence mechanism, one animal of each group was treated with the relatively high dose of 1 in 17,500. The continued treatment of the sub-strain in series H serves as a further important check to the process of drug-resistance in series E and F.

Enumerations were made of the trypanosomes in the animals of the first passage of the experiments recorded in Table I, and these are shown in fig. 1.

The experiments shown in Table II were designed to ascertain how far resistance can be enhanced by a single dose. The transfusion of trypanosomes into a second animal which had been splenectomized and treated with copper enabled us to study the effect of a single intensive treatment. In the first rat in which the defence mechanism had been excluded a dose of germanin equal to 4 mgm. per 100 gm. failed to clear the blood of trypanosomes within 26 hours ; in the second rat, the exclusion of the defence mechanism prevented the destruction by the phagocytes of the parasites, although these were subjected to the influence of a large dose of germanin.

To test the degree of drug-resistance, the trypanosomes were transferred to mice which were treated subcutaneously with germanin. The sterilizing dose signifies no relapse during observations for four weeks.

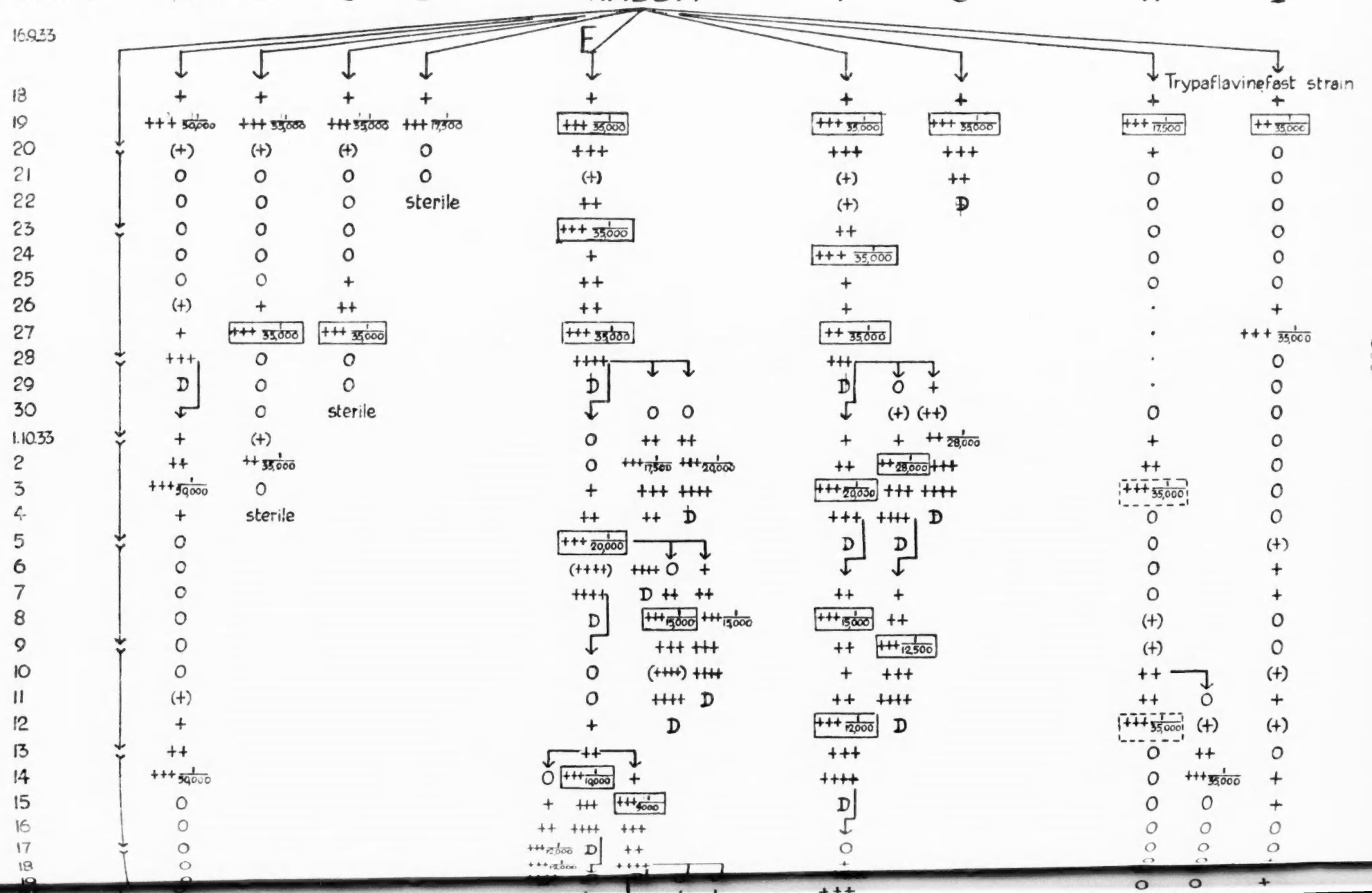
ANALYSIS OF THE EXPERIMENTS

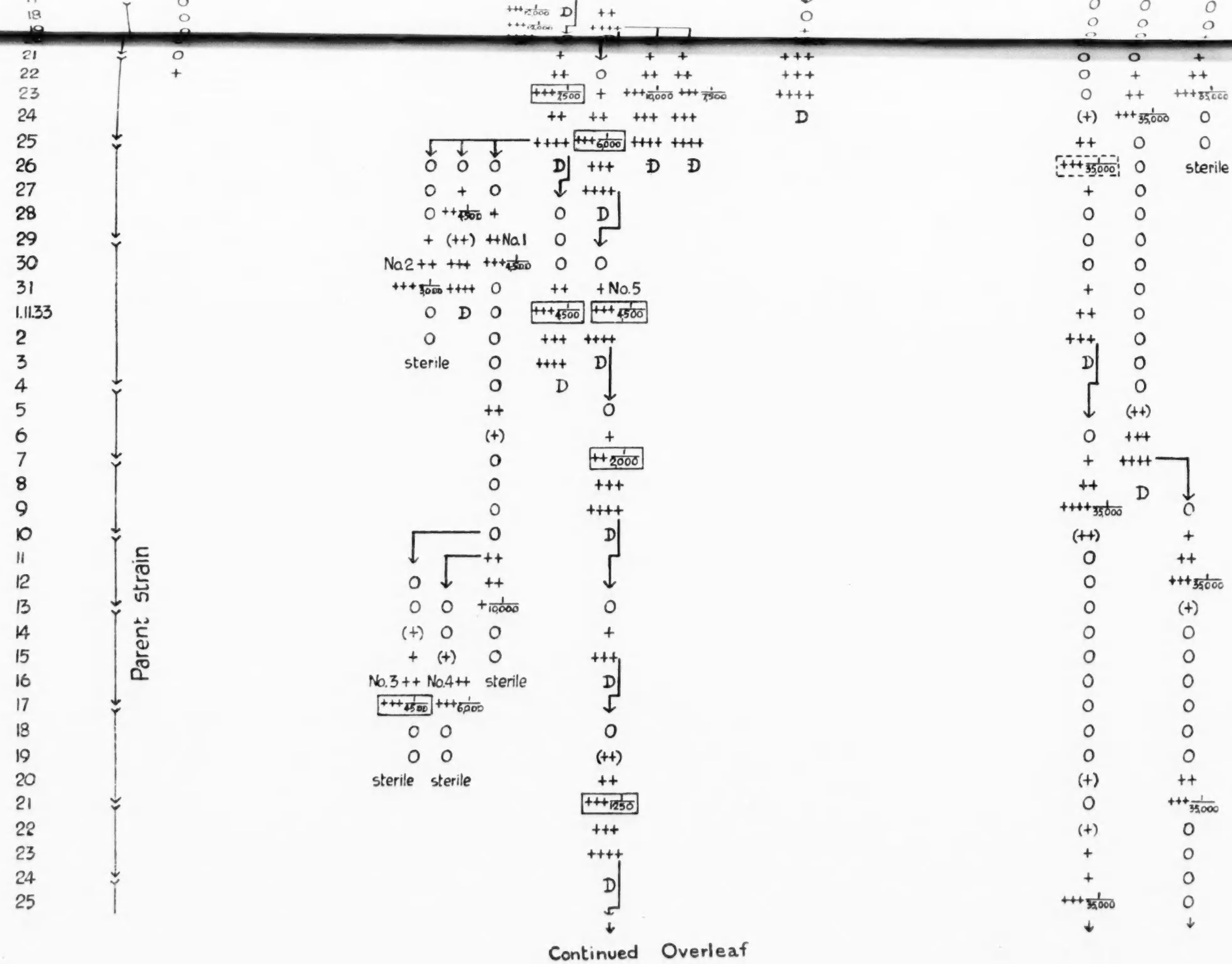
It is clear from the experiments recorded in Table I and fig. 1 that trypanosomal infections in animals in which the biological defence mechanism has been excluded respond to germanin very differently from similar infections in normal animals.

1. The effect of a single dose of germanin is considerably diminished in the ' blocked ' animals (fig. 1) ; a dose of 1 : 35,000 failed to cause the disappearance of the trypanosomes from the blood, and double this dose failed to effect a definite cure.

Trypanosoma brucei "Prowazek" strain

Infection 15.7.33




Parent strain
Continued Overleaf

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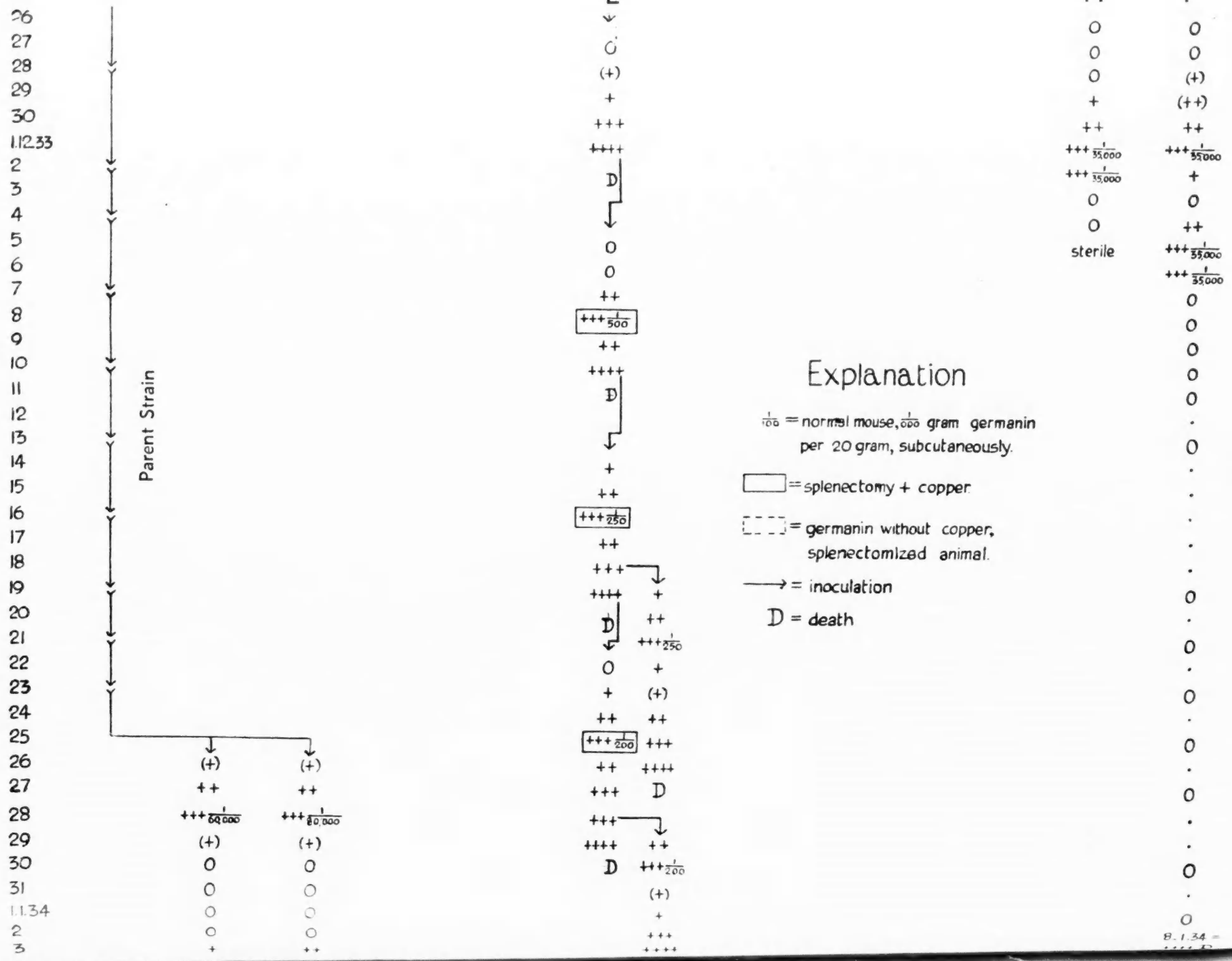
Parent Strain
E
G
(+)
+
+++
++++
D
0
0
++
+++ 1/500
++
++++
D
+
++
+++ 1/250
++
+++
++++
D
0
+
++
+++ 1/200
++
+++
++++
D
(+)
++
+++ 1/80,000
(+)
0
0
+
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+++ 1/80,000
(+)
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0
0
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+++ 1/35,000
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sterile
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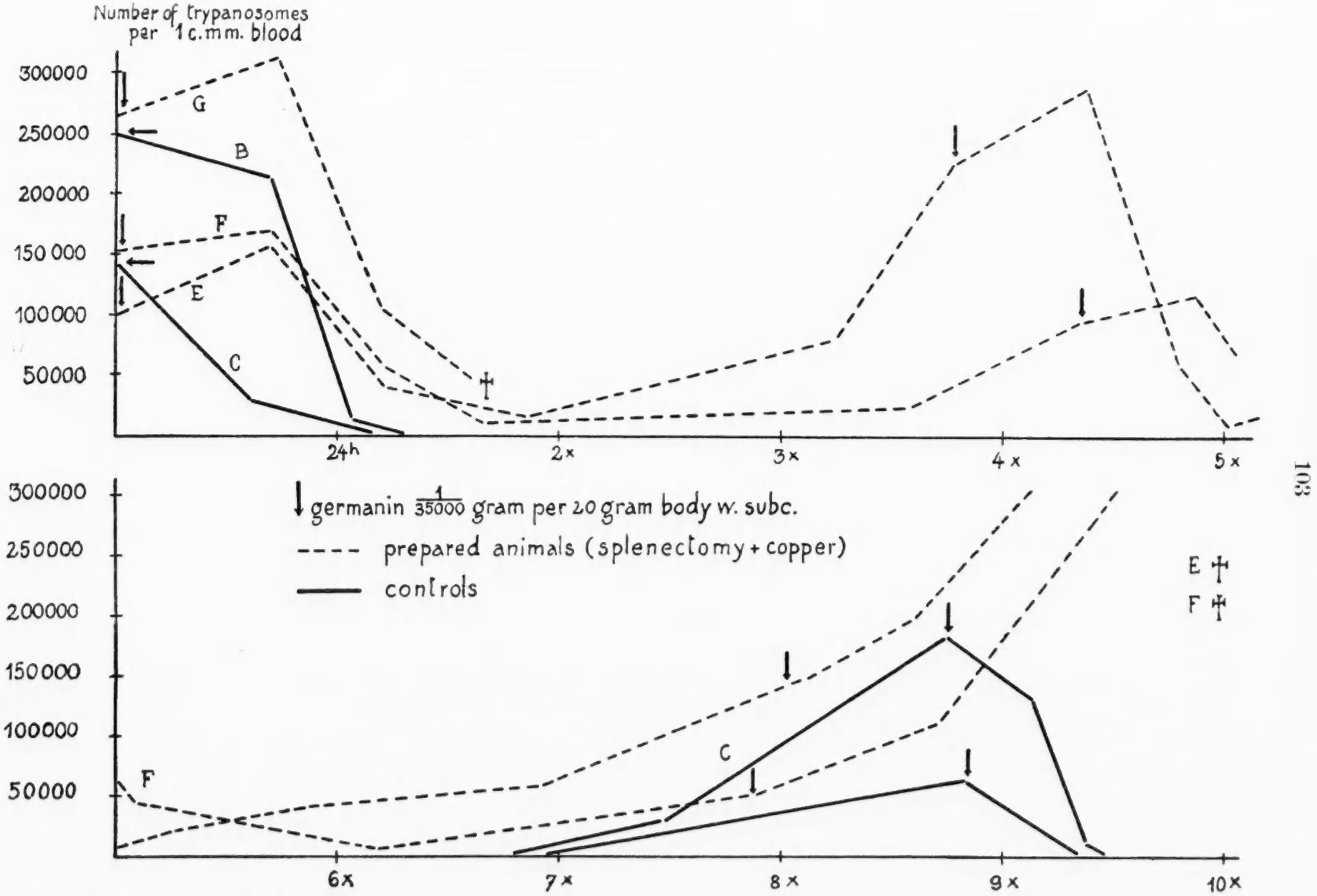

FIG. 1. Behaviour of the trypanosomes in the first passage, series B, C, E, F and G (cf. Table I). Rapid development of germanin-resistance in animals splenectomized and treated with electro-colloidal copper.

TABLE II

Trypanosoma brucei, arsenic-fast strain

Effective dose = $\frac{1}{15000}$ gram germanin subcutaneously per 20 gram mouse.
Curative dose = $\frac{1}{10000}$ to $\frac{1}{9000}$ gram per 20 gram mouse.

Rat No. 1.
(body weight 100 gram)

20.IV.34 — blood +++ trypanosomes
splenectomy, germanin 0·004 gram subcutaneously.
0·4 c.c. colloid copper sol (1+4 water), intravenously.

21.IV.34 (after 26 hours) — +++
Transfusion of the (heparinized) blood
1 c.c. → Rat No. 2 (Splenectomized and treated with copper, 0·4 c.c. intravenously (body weight 100 gram))
into
1 c.c. → Rat No. 3 (Normal) (body weight 100 gram)

	Rat No. 2	Rat No. 3
22.IV.34	++	(+)
23	++	0
24	+++	0
25	+++ germanin 0·001 gram subcutaneously	0
26	+++	0
27	++++	0
28	++++ Mouse 3 / Mouse 4	0
29	++++	0
30	D	0
1.V.34		0
2		0
3		0
4		0
5		0
6		0
7		0
8		0
9		0
10		0
11		0
12		0
13		0
14		0
15		0
16		0
17		0
18		0
19		0
20		0
21		0
22		0
23		0
24		0
25		0
26		0
27		0
28		0
29		0
30		0
31		0

Mouse 1 / Mouse 2

Mouse 1	Mouse 2
+	+
++	++
+++ $\frac{1}{10000}$	+++ $\frac{1}{9000}$
(+++)	++
++	(++)
++++	+++
D	++++
	D

Mouse 1 → Mouse 5

Mouse 5
++
+++ $\frac{1}{2000}$
++
+
(+)
++
+++
++++
D

Mouse 2 → Mouse 6

Mouse 6
+
++
+++ $\frac{1}{3000}$
++
++
+++
++++
D

Mouse 5 → Mouse 7

Mouse 7
++
+++ $\frac{1}{2000}$
++
++
+++
++++
curative dose
D > $\frac{1}{2000}$

Mouse 3 / Mouse 4

Mouse 3	Mouse 4
++	++
+++ $\frac{1}{2000}$	+++ $\frac{1}{1200}$
+++	++
++	(+)
+++	(+)
++++	(+)
D	++
	+++ $\frac{1}{1200}$

Mouse 3 → Mouse 8

Mouse 8
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
curative dose
+++ > $\frac{1}{500}$

Rieckenberg strain + = relapse strain

	Mouse 9	Mouse 10
++		
+++ Germanin 0·001 gm. subcut.	+	+
	++	++
	+++ $\frac{1}{10000}$	+++ $\frac{1}{10000}$
	(+)	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
15.VI.34 = 0	5.VI.34 = relapse	6.VI.34 = relapse
	8.VI.34 = D	8.VI.34 = D
	No marked increase in resistance	

2. When a strain of trypanosomes is subjected to a series of doses of germanin in animals in which the biological defence mechanism has been excluded, the trypanosomes acquire a rapid resistance to the drug ; after 12 such treatments in ' blocked ' animals, the parasites are found to withstand the ' dosis bene tolerata,' i.e., 1/200 gm. of germanin per 20 gm. mouse.

The fact that the therapeutic effect of germanin in prepared animals is much less than in normal animals is due partly to the absence of ' ictus immunisatorius,' but chiefly to the elimination of the phagocytes as the result of the copper injections. The efficacy of germanin in the presence of an intact reticulo-endothelial system is largely due to its opsonizing action ; when once the phagocytes have been excluded, the humoral trypanocidal action alone can play its part. This explains the observation that in the prepared animals germanin exerts its trypanocidal effect relatively slowly and only after a temporary multiplication of the trypanosomes has occurred (fig. 1).

The remarkable speed with which a germanin-fast strain was produced in these prepared animals is in striking contrast to the results obtained by previous workers. Morgenroth and Freund (1924) refer to ' fairly protracted experiments,' and Yorke and his co-workers (1932) record that 12 months was required to make a strain of *T. rhodesiense* completely resistant to germanin. The results of Leupold (1924) are particularly interesting in this connection, because she worked with the same strains as we used in these experiments. From Leupold's results, which are summarized in Table III, it appears that

TABLE III

Strain	1st passage		10th passage	20th passage	30th passage	50th passage	100th passage
	Relapse	Cure	Relapse	Relapse	Relapse	Relapse	
Prowazek	1/50,000	1/40,000	1/20,000	1/14,000	1/2,000	1/2,000	1/150 no effect
Arsenic-fast	1/20,000	1/14,000	1/14,000	1/6,000	1/5,000	1/800	?

maximal resistance to germanin was not obtained until after 100 passages. Our experiments with normal animals are in harmony with these findings (Table I, series B, C and I). The extraordinary speed with which germanin-fastness develops in a series of prepared animals is shown in the following experiment, where it is found that resistance in successive animals increases by geometrical progression with a factor of 2. At the beginning of the experiment

the infection could be healed temporarily by a dose of 1 : 50,000. Assuming the figure to be 1 : 51,200, we obtained the following results :—

Calculated :

51,200 25,600 12,800 6,400 3,200 1,600 800 400 200

Found :

Therapeutic drug passage :

1 2 3 4 5 6 7 8 9 10

Dose in case of excluded defence :

35,000 20,000 9,000 6,000 4,500 2,000 1,250 500 250 200

Afterwards drug-fast to :

20,000 12,000 7,500 4,500 2,000 ? ? ? 250 200

The same fact is brought out by the experiment recorded in Table II. Whereas in mice infected with the normal strain a dose of germanin 1 : 15,000 always causes a temporary disappearance of parasites and a dose of 1 : 10,000 usually produces a permanent cure, in mice infected from a prepared rat which had been given only a single dose of germanin, the infection resisted a dose of 1 : 2,000, and in the mice inoculated from the prepared rat after it had received two doses of germanin a dose of 1 : 500 failed to cure.

A direct relationship must perforce be assumed to exist between the rapid development of germanin-resistance in the prepared animals and the exclusion of natural defence mechanism. Only one other explanation seems to be possible, and that is that in some way or other the copper catalyzes the production of drug-fastness ; the only point in favour of this is the fact that copper is a powerful catalyst. In order to exclude the chemical explanation, it would be of interest to ascertain experimentally how far splenectomy alone influences the rate of production of drug-resistance, but unfortunately lack of animals has hitherto prevented us from examining this question. The researches we have so far made on the mechanism of the production of drug-resistance have led us to the following conclusions.

Our *in vitro* experiments with germanin-fast trypanosomes have shown that resistance to germanin is due to some change in the constitution of the trypanosomes, so that the specific action of the drug is no longer seen in the animal body. The resistance in this particular case is, however, not merely the resultant of the specific parasitotropic action of the drug and of the tendency to habituation on the part of the parasite. These two factors alone will not suffice to explain all the phenomena seen in the production of germanin-resistance. The rapid production of resistance which occurs when the natural defence mechanism is excluded can only be explained on the ground that the parasites tend to adapt themselves very quickly to the action of the drug. That such a quick adaptation does not occur under normal conditions must be due to the intervention of a third factor which inhibits or counteracts the tendency to adaptation by the parasites. As splenectomy and injection of colloidal copper excludes the natural

defence forces, we must also suppose that the production of drug-resistance is inhibited by these very forces.

According to this conception the development of resistance is the result of three factors, viz. : the specific effect of the drug on the parasite, the tendency to adaptation on the part of the parasites, and the inhibition on this process of adaptation exercised by the natural defensive process of the host. If these assumptions are correct, we have here to do with a hitherto unknown function of the defensive forces, the importance of which forces for therapy is clearly shown in our experiments.

Let us now turn to the question as to which of the natural defence powers are concerned with this important rôle in evolution of drug-resistance and how the mechanism of its action can be explained. Whilst our investigations of these problems are not yet complete, we might refer to an important observation recorded by us in Table I, series E, which suggests that rapid adaptation by the parasites is being counteracted by specific antibodies. In this series it is seen that mouse no. 1 was infected with trypanosomes which had already been made sufficiently resistant to withstand 1 : 4,500 of germanin, although, as mouse no. 2 shows, 1 : 3,000 effected a permanent cure. Now when mouse no. 1 relapsed we tested the sensitiveness of the relapse strain in this mouse and in two subinoculated mice, no. 3 and no. 4. The results show that the previous degree of resistance had decreased, because 1 : 6,000 and 1 : 10,000 now produced permanent cures in the normal animals and 1 : 4,500 in the prepared animal, no. 3.

This observation seems to afford support to the hypothesis that in normal animals immune bodies play a part in the slow development of resistance. It may well be imagined that the considerable alterations which must take place in trypanosomes during the development of a relapse strain may be associated with changes in the reverse direction of their adaptation to a drug. It may also be surmised that antibodies arising from a single treatment may interfere primarily with the process of adaptation. By excluding the defence forces of the host free play is given to the process of adaptation of the parasites.

The humoral forces are in the main excluded by splenectomy, but it is still a matter of speculation as to what could be obtained from splenectomy alone unaided by the copper medication. Splenectomy alone hardly affects the acute curative process. Our experiments have demonstrated that it was only after the elimination of the phagocytes by the electro-colloidal copper that a large number of trypanosomes survived the powerful chemotherapeutic shock. This procedure thus on the one hand increases the probability that specially reactive individuals will quickly acquire drug-resistance in a blood rich in germanin, and on the other hand the possibility of a relapse strain is entirely excluded owing to the lack of immune bodies.

Our hypothesis that the immune bodies are those factors of the defence forces which exert an inhibiting influence on the development of resistant

trypanosome strains is supported by certain earlier observations of other workers indicating that a similar regression of drug-resistance follows the formation of relapse strains, as we ourselves have observed in the case of germanin. Ritz (1914) states that a strain of trypanosomes absolutely resistant to trypan-blue when treated with subtherapeutic doses of neoarsphenamine produced relapse strains fairly sensitive to trypan-blue. The loss of drug-resistance shown by relapse strains was also described by Ehrlich (1909) for trypan-red, by Morgenroth and Rosenthal (1912) for their hydrochinin-fast strains, and by Schnitzer and Silberstein (1928) for parafuchsin-fast strains.

SUMMARY

1. Since the natural defence forces play an important rôle in the curative effect of chemotherapeutic agents, especially of germanin, we considered it important to ascertain whether development of germanin-fast *Trypanosoma brucei* strains in prepared mice and rats is influenced by the exclusion of the natural mechanisms of defence, and, if so, to what extent.

2. In mice, exclusion of the biological defence forces was effected by splenectomizing the animals 2 to 4 hours before treatment, and then by injecting electro-colloidal copper intravenously 3 or 4 hours after the injection of germanin.

3. When infected animals in which the biological defence mechanism has been excluded, as described above, are treated with germanin, a drug-fast strain is obtained with remarkable rapidity, whereas, as is well known, the production of a germanin-fast strain by the ordinary method is a very long matter.

4. In splenectomized mice treated with copper, strain ' Prowazek ' became resistant to the well-tolerated dose after only twelve successive doses. This implies an increase in resistance 250 times that of the normal. With the usual technique, nearly 100 therapeutic drug-passages would be necessary to obtain a similar resistance in this strain.

5. In a second strain, an increase of resistance of more than seven times that of the normal was found in a prepared rat after a single treatment.

6. The most plausible explanation appears to be that trypanosomes possess the capacity of adapting themselves very quickly to the drug, and that normally a rapid production of drug-fastness does not take place because the defence forces counteract the tendency to do so.

7. The therapeutic significance of this function of the defence powers is apparent from the fact that treatment of a trypanosome infection with repeated non-sterilizing doses led in normal mice to a permanent cure, whereas, after the exclusion of the protective forces, the same treatment was rendered totally ineffective by the rapid production of drug-resistance.

8. The fact that, after a relapse in a normal mouse, trypanosomes may again become sensitive to germanin suggests that relapse antibodies may materially influence the process of production of drug-fastness.

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